Contract for:

INTEGRATED PROJECT

Annex-I – “Description of work”

Project acronym: NEUROPROMISE
Project full title: Neuroprotective strategies for multiple sclerosis
Proposal no.: 018637
Related to other Contract no.: 

Date of preparation of Annex 1:

Start date of contract:
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1. PROJECT SUMMARY

Despite intensive research on the causes and pathogenesis of multiple sclerosis (MS), the genetic basis of this common cause of neurological disability is still unknown and the complex immunopathological mechanisms underlying brain injury are only beginning to be understood. To date, no curative therapy is available and none of the existing drugs can stop the neurodegenerative process responsible for disease progression. The NeuroproMiSe project represents a highly focussed and integrated effort to investigate in depth the genetic and mechanistic pathways involved in inflammation-induced neurodegeneration, and exploit this knowledge to develop novel candidate drugs for effective neuroprotective therapy.

NeuroproMiSe specific objectives are: 1) To identify the major genes and critical pathways associated with MS and inflammatory neurodegeneration. This goal will be achieved through a comparative genetic approach, using an established animal platform suitable for identification of EAE susceptibility genes, and well-characterized, ethnically homogeneous cohorts of MS patients. Through genomic and proteomic screens carried out in animal disease models, further knowledge will be obtained on genes and molecular pathways involved in inflammatory neurodegeneration. 2) To elucidate essential immunopathological mechanisms of neurodegeneration, focussing on innate and adaptive immune responses and on the use of humanized animal models to define new rational ways of developing disease modifying drugs. 3) To develop novel neuroprotective drugs based on targets validated in animal models. In a first step, new therapeutics targeting critical pathways (Ncf-1, TREM-2, TNFR subtypes, ion channels, glutamate receptors, apoptosis) identified by the applicants, will be tested and used in combination therapies. The project is conducted in alliance with four biotechnology companies, 3 of which are SMEs, and has potential for direct transfer of basic research results into clinical studies and economic and society benefits.
2. PROJECT OBJECTIVES

The overall aim of the NeuroproMiSe project is to elucidate the molecular mechanisms underlying inflammation-driven injury of the central nervous system (CNS) and to define novel targets, validated in animal models, for the development of therapeutic strategies for highly debilitating neuroimmune diseases. NeuroproMiSe will achieve this goal through a disease-oriented approach by identifying the essential genes and critical pathways leading to multiple sclerosis (MS), the most common inflammatory disease of the CNS. Given the importance of axonal damage and neuronal loss in determining clinical deterioration in MS patients and the lack of effective therapies for prevention of MS progression, the project is strongly oriented towards achieving a better understanding of the molecular basis of neurodegeneration, and direct protection of axons/neurons through targeting of critical signaling pathways. The knowledge and products generated in this project may also have implications for the pathogenesis and therapy of other acute and chronic neurodegenerative diseases with an inflammatory component.

The major, specific NeuroproMiSe objectives are the following:

1- To identify candidate genes involved in CNS tissue destruction in MS and animal models of neuroinflammation and neurodegeneration, and decipher their pathophysiological role. This goal will be achieved through:

i) A ‘disease-driven’ strategy, that will allow to identify genes and pathways associated with disease susceptibility and course in animal models of MS and neurodegeneration, and to use this information to define synonymous genes in homogeneous and well characterized cohorts of patients with MS and population-based controls. The unique animal platform of mouse and rat congenic strains established by P3 and P8 will be used for identification of disease-susceptibility genes and associated molecular pathways (first results expected by month 18), and for the study of gene-gene and gene-environment interactions to identify new disease-regulatory genes and their interactions (month 36). Validation of candidate genes and mechanistic pathways will be performed by gene and protein expression analysis and bioinformatics, and by using a wide array of already-existing and to-be-developed genetically modified mice in relevant disease models (knockin, knockout and knockdown) (month 24 to 60). These animal models will be also used to test the efficacy of neuroprotective compounds (up to month 60).

ii) Identification of disease-associated genes in MS case cohorts and families, exploiting the knowledge derived in experimental disease models. The human studies, which will be conducted in parallel with the animal studies, will utilize genomic DNA from a large cohort of Swedish MS patients and ethnically matched controls (P8), and families affected by MS in Finland (P9), all with extensive clinical and genotypic characterization. In a first step, new genotypic information will be obtained in the context of already known risk genes, to control for epistasis and other gene-gene interactions (month 18). To characterize the allelic diversity of these known genes, high-throughput single nucleotide polymorphism (SNP) genotyping and haplotype analysis will be carried out (up to month 24). SNP genotyping will be then performed for selected genes identified in the animal model genetic platforms (up to month 60). In an attempt to link disease-relevant gene polymorphisms to specific patterns of CNS tissue damage, a smaller cohort of MS patients with extensive neuropathological information obtained from brain biopsies (P2b) will be included in these studies.

iii) Application of advanced genomics and proteomics to animal models of neuroinflammation and neurodegeneration (including myelin mutant mice, TREM2/DAP12 deficient mice, Wallerian neurodegeneration) to identify new targets for neuron- and axon- protective therapy as well as biomarkers of early and late signs of neuronal loss (month 24). Candidate genes/proteins will be confirmed using different approaches (real time PCR, in situ hybridisation, immunohistochemistry, western blotting) (month 36) and tested for pathophysiological relevance using gene targeting strategies and functional assays (up to month 60). The data obtained in the different models will be compared with the aim of...
identifying common genes or pathways that contribute to neurodegeneration (month 36). These animal models will be also used to validate potential therapeutic interventions in more advanced stages of the project (up to month 60).

2- The second objective of NeuroproMiSe is to analyse essential immunopathological mechanisms of axonal and glial injury in MS, to support the development of new protective therapies for neuroimmune disorders. To obtain further insight into the specific involvement, relevance and interdependence of different immune-mediated mechanisms of CNS tissue damage, the following tasks will be addressed:

i) improved transgenic animal models for MS with inducible and constitutive expression of a specific antigen in defined neuronal and glial cell populations will be developed by P6 to identify in vivo the mechanisms of CNS tissue damage mediated by cytotoxic CD8+ T cells (month 24);

ii) humanized animal models will be generated to study the role in MS-like disease evolution of autoreactive human CD8+ T cells recognizing an HLA:neural self peptide complex relevant for MS or cross-reactive with microbial antigens, and of epistatic interactions in the MS-associated DR2 haplotype (P4) (month 24);

iii) the pathogenic relevance of ectopic lymphoid tissue (B-cell follicles) in neuroinflammatory processes will be investigated by P1 in the MS brain and in EAE models using strategies to promote or prevent the formation of an intracerebral lymphoid microenvironment (month 24);

iv) in vitro and in vivo models will be developed by P19 to study the signalling pathways underlying axonal transport disturbance and axonal injury induced by microglia-derived inflammatory mediators (month 24).

The above studies are expected to generate new knowledge on MS immunopathogenesis (month 36), which will help to define new rational ways of developing disease modifying drugs, and new animal models useful for analysing interactions with newly identified genes and for evaluating the therapeutic efficacy of neuroprotective compounds developed by other participants (up to month 60).

3- The third objective of NeuroproMiSe is to develop new neuroprotective drugs based on targets identified and validated in animal models. As a first step, a number of critical pathways of neurodegeneration and neuroprotection will be investigated, and already existing and newly developed drugs will be tested, in search of the best strategy to protect CNS cells from inflammatory insults. Targets identified in the genetic and genomic studies and validated in the disease models described above will also provide seeds for the development of new therapeutic compounds. In this context, the specific tasks will be:

i) to achieve neuroprotection by targeting molecules that are critically involved in the regulation and function of myeloid cells, like the TREM-2 receptor and its associated signalling molecule DAP-12, and Ncf-1, a component of the NAPDH oxidase complex. New compounds targeting the Ncf-1 pathway will be developed by P17 (month 18). The molecular pathways regulated by TREM-2 and Ncf-1 will be further explored (up to month 48). Moreover, compounds modulating TREM-2 and Ncf-1 pathways will be evaluated for their therapeutic efficacy in animal models of CNS inflammation (up to month 60).

ii) to develop compounds targeting tumor necrosis factor receptor (TNFR) subtypes for neuroprotective therapy (month 24). Based on previous technology developed by the applicants (P12, P13, P16 and P20), genetic engineering of human TNF and antibody-based reagents will be used to construct novel TNFR specific agonists and antagonists capable of a selective triggering of TNFR2 and a selective inhibition of TNFR1, respectively (month 24). Genetically modified mice that express the two human TNF receptors will be generated by P13 to investigate the in vivo function of human specific therapeutics targeting TNF receptors (month 24). In a complementary approach, P14 will explore a recently identified mechanism of neuroprotection, which involves the TNFR1 intracellular signalling pathway in CNS neurons and is effective in acute excitotoxic and ischemic brain injury, and use this information to develop cell penetrating compounds that will enhance this neuroprotective pathway (month 24). The therapeutic efficacy of the above compounds will be tested in in vitro systems, humanized animal models and other models of neuroinflammation/neurodegeneration utilized by the
iii) to evaluate the efficacy of: a) sodium channel blockers and b) calcium channel blockers and glutamate receptor antagonists, in axonal protection. New drugs that block different types of sodium channels, inhibit the reverse mode of operation of the sodium/calcium exchanger and counteract the neurotoxic actions of glutamate receptor activation leading to excessive accumulation of cytosolic calcium will be tested by P11 and P18 in experimental systems of increasing complexity (month 36). This should help identify the best compound to be used in clinical trials (month 48 or 60).

iv) to evaluate the therapeutic efficacy of combined anti-apoptotic and anti-inflammatory compounds in a rat model of EAE with optic neuritis. Specific intracellular pathways involved in neuronal apoptosis will be manipulated by treatment with neurotrophic factors, neurotrophin-like substances, pharmacological approaches or new protein transfer tools (TAT-fusion proteins) developed by P2a (month 36). These tools will be also tested in combination with known immunomodulatory agents and compounds developed by other partners (up to month 60).

The generation of new compounds, tested for their anti-inflammatory and neuroprotective efficacy, and of bifunctional molecules for targeting of neuroprotective activities specifically to neurons and glial cells will permit the application of innovative approaches for the treatment of neuroinflammatory diseases. The simultaneous development and complementarity of the strategies described above ensures that substantial interactions and synergism among participants will take place during the entire project and will lead to new insights into the mechanisms underlying inflammatory neurodegeneration and neuroprotection, opening the way to the evaluation of combination therapies in clinical trials.

To accelerate the achievement of the NeuroproMiSe objectives, horizontal activities will be developed through the establishment of neuropathology and genomics/proteomics facilities (month 1 or 7 to 60) and training activities (month 10 to 60). This will ensure the uniform availability and distribution of technological know-how in cutting-edge technologies of lesion analysis/imaging and functional genomics.

The NeuroproMiSe management will be implemented through a General Assembly and a Steering Committee, which will oversee all scientific, training, innovation and administrative activities of the consortium (month 1 to 60). To support coordination, training and result dissemination, a NeuroproMiSe interactive web-site will be created and continuously updated (month 6 to 60).

The NeuroproMiSe partners hold all the technologies and patents relevant to the strategies pursued in this project and all partners have freedom to operate within the outlined project. Within the consortium, collaborations between different groups already exist with a long history of successful cooperation. The global knowledge and expertise of the partners in human and animal genetics, clinical and experimental neuroimmunology, and neurobiology, and the participation of SMEs with a strong interest in the development of anti-inflammatory and neuroprotective drugs, constitute the ideal framework for the success of this project and will stimulate cross-disciplinary research and training, as well as promote innovative solutions. It is expected that with the knowledge acquired about specific genes and pathways involved in inflammatory neurodegeneration and neuroprotection, and with the compounds developed on the basis of this knowledge, within the 5 years of the project candidate molecules will be made available and proposed for subsequent clinical evaluation. Altogether, the planned activities of the NeuroproMiSe consortium will represent a significant advance of the state of the art. Knowledge and products generated will speed up effective treatment of neuroimmune diseases.
### 3. LIST OF PARTICIPANTS

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4. RELEVANCE TO THE OBJECTIVES OF THEMATIC PRIORITY 1: LIFE SCIENCES, GENOMICS AND BIOTECHNOLOGY FOR HEALTH

All aspects of the NeuroproMiSe Project are aligned with the specific topic ‘Combating major diseases: Neuroimmune disorders: From basic to clinical research’. Particularly, the overall aim to identify genetic risk factors, understand the pathophysiology and provide new bio-therapeutic compounds to cure chronic and highly debilitating neurological diseases for which no effective treatment is still available is addressed in this Integrated Project.

Combating neuroinflammatory diseases is a major social and economic task. In recent years, it has become increasingly clear that in a variety of CNS diseases, including MS, infectious and post-infectious encephalitis/encephalomyelitis, neurological complications of systemic autoimmunity, Rasmussen encephalitis, stroke and traumatic brain injury, the inflammatory process is closely associated with damage to axons and neurons, and that neuronal loss is the major cause of progressive neurological impairment. MS is the most common cause of neurological disability in young and middle-aged adults, with the highest prevalence rates being observed in Northern and Central Europe (> 30 cases per 100,000 population). Neuroimmune disorders have a high economic burden on the society, since brain inflammation is associated with numerous significant disabilities, including motor and sensory deficits, loss of cognitive functioning, attention, language, incontinence, insomnia, and behavioral problems.

Centres in Europe and in particular the biomedical industry recognize that understanding the molecular mechanisms of neuroinflammation and neurodegeneration will open new avenues for the development of novel therapeutics. However, the search for new therapeutic targets of neuroinflammation requires a systematic collection of disease-afflicted material from humans and experimental models as well as access to the recently available high throughput genotyping, genomic and proteomic screening technologies. Furthermore, the identified targets must be validated in relevant preclinical models of neuroinflammation and appropriate therapeutic tools need to be generated in close collaboration with the pharmaceutical industry. Within the NeuroproMiSe project, there are several European laboratories with strong expertise in animal and MS genetics, basic neuroscience, neuropathology, immunopathology of neurological diseases and establishment of animal models, and industrial partners with a strong interest in developing therapeutics for inflammatory and neurological diseases. This consortium will integrate their research potential to identify susceptibility genes and gene-environment interactions in carefully selected cases and families with MS, and, by transcriptome and proteome analyses, new molecules and pathways involved in inflammatory neurodegeneration. The consortium will validate their role in in vitro and in vivo experimental models and, based on this knowledge, will develop new therapeutic compounds. New, genetically engineered mouse models will be generated that represent invaluable tools for both basic research and disease modelling; these animal models will be available to the scientific community outside the consortium. By detailed analysis of human material, relevant disease models and pre-clinical therapeutic trials, and by exploring the involvement of both the innate and adaptive immunity arms in mediating CNS tissue destruction, the NeuroproMiSe project also aims to provide new insights into inflammatory mechanisms responsible for neuronal and glial cell damage and to define biomarkers for evaluation of pathogenic processes and responsiveness to therapeutic treatments. Moreover, the project will use anti-inflammatory and neuroprotective compounds (i.e. compounds modulating TNFR subtypes and intracellular pathways, TREM-2 signaling and oxidant pathways, anti-apoptotic TAT-fusion proteins and ion channel blockers), recently generated in the applicants’ laboratories, in the attempt to develop novel and combined therapeutic strategies to prevent inflammation-driven neurodegeneration. Although a large part of the work will be performed in MS patients and in animal models of MS, it is likely that the knowledge and tools generated will also enhance understanding of pathogenetic mechanisms and allow development of new therapeutic approaches in other acute and chronic neuroinflammatory disorders.

The scientific, technical and methodological contributions of the project NeuroproMiSe are coherent with the overall focus of the LifeSciHealth Priority within the 6th FP. This Integrated Project as a whole focuses on translational research and uses basic and functional genomic and proteomic knowledge for...
translation into therapeutic concepts and further into preclinical application. Moreover, the application of genetic engineering and nanotechnology will permit the generation of new classes of highly specific reagents targeting the CNS. In this respect the project will contribute substantially to the priority area 'Genomics and Biotechnology'. NeuroproMiSe will contribute towards the improvement of quality of life and health and will reduce the costs for the treatment of patients affected by neuroinflammatory diseases.

The NeuroproMiSe consortium provides a ‘unique training resource’ for European researchers. It encompasses groups with a constellation of complementary expertise from multiple disciplines (e.g. clinical neurology, neuropathology, human and animal genetics, neuroscience, immunology, molecular and cellular biology, biochemistry, electrophysiology, nanotechnology, experimental disease models and state-of-the-art technologies in functional genomics), working in collaboration with industrial partners, and represents the ideal context for training in all aspects required for attaining the project objectives. Thus, the training of scientific and technical personnel will constitute an essential objective of the NeuroproMiSe project activities (courses, workshops, laboratory stages) and will give a significant added value. The availability within the consortium of Neuropathology, Genomics and Proteomics Reference Centers will not only facilitate training but also allow all the NeuroproMiSe partners to have access to relevant pathological material and count on appropriate support and expertise for pathological analyses and high throughput technologies.

The NeuroproMiSe consortium will also contribute to cohesion in the EU. The visits of scientists belonging to the consortium, for exchange and/or training, will foster European integration. An additional advantage is represented by the establishment of long-lasting collaborations that will further promote and amplify the integration process well beyond the duration of the present program and implement the scientific excellence of the participating groups and of EU in general.

Finally, the NeuroproMiSe consortium will contribute to strengthen the competitiveness of the small/medium European industry. In total, 3 out of 19 partners are SME’s, and they account together 9.6% of the requested funding. SMEs are actively participating both in scientific and training activities and lead some of the scientific workpackages. The contribution of SMEs also guarantees an efficient approach in the dissemination and exploitation of the results achieved within the NeuroproMiSe project.

State of the art in MS and neuroinflammation research and new expected achievements of the NeuroproMiSe project

Genes involved in MS susceptibility and neurodegeneration

MS is a complex disease, in which complexity is manifested at different levels, including genetic background, pathogenesis, clinical presentation, and response to treatment. MS exhibits several characteristics in common with other chronic inflammatory diseases, like rheumatoid arthritis (RA), including polygenic inheritance, partial susceptibility conferred by HLA-associated genes, and influence of environmental factors. Due to this complexity, family-based linkage analyses and population-based association studies have until recently failed to consistently identify MS regulating genes outside the human leucocyte antigen (HLA) complex. There are at least three different reasons for this: 1) genetic heterogeneity, e.g. different individuals/families/populations may have different disease predisposing genes; 2) small or modest effects by individual genes; and/or 3) lack of statistical power owing to the small size of the studied patient cohorts. Identification of disease-related genes in human complex diseases clearly requires a different strategy. The use of inbred animal strains, in which relevant models for human diseases can be established, can greatly increase the power of identifying genes in complex diseases for several reasons: i) mouse and rat strains are inbred; thus, the genetic segregation can be followed making linkage analysis productive; ii) genes can be isolated by selective breeding, positional cloning and transgenic technology; iii) the environmental influences can be controlled; iv) gene-gene interactions can be analyzed through cross-breeding of congenic strains; v) the genome can be modified, making it possible to test the function of specified gene variants and define the molecular pathways involved; vi) the information obtained in the animal studies can be utilized to identify synonymous genes in humans. This strategy has already proven useful in identifying disease-associated loci in animal models of RA and in showing that approximately 50% of the genetic influence is shared between models of RA and MS, like experimental autoimmune encephalomyelitis (EAE) (work by P3, P8 and...
Histologically, MS is characterized by an accumulation of T cells, B cells and activated macrophages in the CNS parenchyma, overlaying meninges and CSF. This inflammatory process is thought to be sustained by autoreactive T cells and autoantibodies recognizing CNS (possibly myelin) antigens, and by activated macrophages/microglia, and is responsible for demyelination, axonal damage and neuronal loss. Neuropathological studies by the applicants (P2b, P10) have demonstrated that axonal injury is already evident in the early stages of plaque formation and correlates with the presence of CNS-infiltrating cytotoxic CD8+ T cells and activated macrophages/microglial cells (Ferguson et al., 1997; Brain 120:393; Kuhlmann et al., 2002; Brain 125:2202). In vitro studies by P19 have shown that CD8+ T cells are directly implicated in neurodegeneration (Medana et al., 2001; Am. J. Pathol. 159:809) and that alterations in axonal transport can be induced by activated microglia (Stagi et al., 2005; J. Neurosci. 25:352). Work by P4 has provided structural evidence for molecular mimicry (foreign antigen mimicking a self peptide) involving HLA molecules, supporting the idea that infectious organisms can trigger or exacerbate the disease (Lang et al., 2002; Nat. Immunol., 3:940). Heterogeneity of the pathogenic mechanisms involved in MS lesion development has been proposed recently in studies by P2b and P5 (Lucchietti et al., 2000; Ann. Neurol. 47:707). Evidence is also emerging that dysregulated immune processes that remain compartmentalized behind the blood-brain and blood-CSF barriers, like ectopic lymphoid...
structures developing in the inflamed meninges of mice with relapsing EAE and patients with secondary progressive MS (work by P1: Magliozzi et al., 2004; J. Neuroimmunol. 148:11; Serafini et al., 2004; Brain Pathol. 14:164), may contribute to the slow neurodegenerative process associated with chronic neuroinflammation. A deeper knowledge of the molecular mechanisms underlying these detrimental processes is however necessary to clarify their relative contribution in different disease phases and to develop new strategies to counteract them. Through the development of improved animal models for MS (transgenic mice with inducible and constitutive expression of specific antigens in defined CNS cell types; humanized double transgenic mice expressing MS-associated MHC molecules and related TCR; mice overexpressing critical lymphoid chemokines in the CNS) and in vitro models (microglia-neuron cocultures in which candidate deleterious and neuroprotective genes are either knocked down or overexpressed in defined cell types), this project will provide novel information on candidate immune pathways involved in CNS tissue destruction and this will help to define new rational ways of developing disease-modifying drugs. The above models will also aid in evaluating the therapeutic efficacy of neuroprotective compounds developed by other participants.

**Therapeutic strategies in MS.** While an autoimmune attack directed against myelin components is thought to initiate MS lesions and to predominate in the initial, relapsing-remitting phase of the disease, axonal injury and the ensuing secondary neurodegeneration, which determine the accumulation and irreversibility of neurological deficits, seem to prevail in primary and secondary progressive MS. Approved disease modifying drugs for the therapy of relapsing-remitting MS act by regulating or suppressing the immune response and partially reduce clinical relapses and development of new brain lesions, but are totally unable to stop neurodegeneration and disease progression (Fox and Ransohoff, 2004, Trends Immunol. 25:632). Progressive forms of MS currently have no proven effective therapy. Although new strategies targeting lymphocyte homing to the CNS (e.g., chemokine and adhesion molecule antagonists) look promising for MS, a recent phase III trial with a humanized anti-integrin antibody has unravelled the risk of serious adverse effects (deadly-viral brain infection) of such an approach. Thus, the development of novel and safe immunoregulatory compounds remains a priority in MS research. Recently, major attention has focussed on the role of innate immunity in autoimmune diseases (Bach et al., 2004, J. Exp. Med 200:1527) and the discovery of novel molecular pathways affecting the activation state of innate immune cells (in particular dendritic cells, macrophages and microglia) or mediating their functions has opened new perspectives for controlling immunopathological processes (Aoki et al., 2003, Curr. Pharm. Des. 9:7; Olofsson and Holmdahl, 2003,Scand. J. Immunol., 58:155).Within this project, further exploration of two novel immune regulatory pathways (Ncf-1 and TREM-2/DAP-12 pathways) and evaluation of the therapeutic efficacy of compounds targeting these pathways will provide new information on the involvement of innate immunity in neuroinflammation and this knowledge will be used to generate new tools for combined anti-inflammatory/neuroprotective therapeutic strategies.

Because MS can be diagnosed accurately before major neuronal damage occurs, the search for novel therapeutic strategies able to directly prevent neuronal injury is mandatory. So far, only a few attempts have been made to test neuroprotective strategies in animal model of MS and these have mainly involved the use of glutamate antagonists and erythropoietin derivatives. The therapeutic efficacy of combined neuroprotective and anti-inflammatory compounds in EAE and other models of neuroinflammation also need to be evaluated and new protocols have to be established for the concomitant or sequential blocking of inflammatory and neurodegenerative events. Based largely on work performed by the applicants, several hypotheses have been put forward to explain MS-associated neurodegeneration. These include changes in myelin-axonal interactions, disturbed impulse conduction due to abnormal distribution of sodium and calcium channels on the axonal membrane (Kornek et al., 2001; Brain 124:11114; Craner et al., 2004; Proc. Natl. Acad. (USA) 101:8168), ischemic and excitotoxic injury (Lassman et al., 2003; Brain 126:1347; Smith et al., 2000; Nat Med. 6:62), attack of neurons by autoreactive T cells and autoantibodies (Neumann et al., 2002; Trends Neurosci. 25:313), and direct neurotoxic effects of inflammatory mediators (Akassoglou et al., 1998, Am. J. Pathol. 153: 801; Probert et al., 2000; Brain 123:2005). Moreover, recent findings of a molecular segregation of neurodegenerative/proinflammatory activities from neuroprotective activities of TNF signalling, with TNFR2 conveying neuroprotection in models of acute neurodegeneration (work by P12, P13; Fontaine et al., 2002; J. Neurosci. 22: RC216:1; Marchetti et al., 2004; J. Biol.
Chem. 279:32869) and TNFR1 having both inflammatory (P14: Akassoglou et al., 1998; Am. J. Pathol. 153: 801) and neuroprotective (P14: Taoufik et al., submitted) activities provide the basis for the development of new and improved compounds (e.g., blood-brain barrier and cell-type specific penetrating molecules) targeting selectively one or the other TNFR subtype. The generation of new compounds, tested for their anti-inflammatory and neuroprotective efficacy, and of bifunctional molecules for targeting of neuroprotective activities specifically to neurons and glial cells, will permit the application of innovative approaches to counteract inflammation-associated neurodegeneration. By pursuing the above basic concepts, evaluating recently developed compounds with improved antagonistic activity for sodium channels and glutamate receptors, and developing novel tools to modulate TNFR signaling and apoptotic processes in neurons and glial cells, we expect to provide a new generation of effective neuroprotective compounds to be used in patients with MS and, possibly, other neuroinflammatory diseases.

5. POTENTIAL IMPACT

Chronic neurodegenerative diseases have a high and increasing prevalence in the EU and pose a major socio-economical challenge. For many years the relevance of neuroinflammation in neurodegenerative diseases, including MS, has been underestimated. Recently, evidence has emerged that the damage of axons and neurons in MS is responsible for the long-lasting neurological deficits of the patients. Although several disease-modifying drugs with an immunomodulatory/immunosuppressive activity have been recently approved for MS, none of the existing therapies can stop disease progression and accumulation of neurological deficits. Therefore, the search for more effective and selective therapies capable of preventing CNS tissue destruction and permanent neurological disability remains a priority in MS research. One major aim of the pharmaceutical and biomedical industry is to develop drugs to protect neurons and axons against neuroinflammatory damage. The NeuroproMiSe project will enhance the European excellence in this new field with a major impact on the understanding of MS pathogenesis and on the development of therapies targeting novel pathways of neuroinflammation and neuroprotection. Although specifically addressed to MS, it is conceivable that these efforts will also have an impact on other acute and chronic neuroinflammatory diseases. The NeuroproMiSe project will combine the knowledge and expertise of European academic and governmental research centres and industrial partners working in the fields of MS genetics, neuropathology, basic neurobiology and immunology and using distinct model systems for neuroinflammation and neurodegeneration.

The added-value of the Project NeuroproMiSe relies on the following:

- NeuroproMiSe overcomes current fragmentation in the field by integrating the complementary skills of groups from EU Member States that are in the front line of research in neuroinflammatory diseases and basic neurosciences and immunology.
- NeuroproMiSe gathers together a wide array of experts into a highly focussed project. The integration will be reached through the common goal of uncovering the molecular mechanisms of neuroinflammation and of designing relevant therapeutic strategies for effective cure of neuroimmune diseases. Specialists in the above-mentioned areas will work together to create critical mass, synergy and added-value.
- NeuroproMiSe promotes innovative results through their adoption by industry, in the form of technological transfer, triggering collaborations and favouring spin-off companies, where appropriate.
- NeuroproMiSe equalizes the flow - between the USA and Europe - of researchers. In the virtuous cycle of healthy competition, it should make European research equally attractive for US teams.
- NeuroproMiSe performs education and training activities to transfer state of the art knowledge to EU students and researchers. The objective is to define and promote integrated European curricula on molecular and cellular neurosciences, neuronal cell death mechanisms, neuroprotection, neurogenetic-based clinical studies, and neuroimmunology. Attention will be
also paid to transfer of knowledge to patients, health actors and providers, as well as to the common population, taking into consideration all ethical consequences of the techniques or therapeutic strategies available to date.

- NeuroproMiSe will support international collaboration with an aim to open European research to leading teams and projects in the area and to stimulate fruitful competition.

Listed below are the innovative aspects of the NeuroproMiSe project that go beyond the state of the art:

1) The availability of material collected from large cohorts of well-characterized and ethnically homogeneous MS patients and of unique animal platforms and animal models of CNS inflammation and neurodegeneration, to be utilized for a comparative genetic approach aimed to the identification of genes involved in inflammatory neurodegeneration.

2) The availability of biopsy brain material from a small, but well characterized, number of MS patients to be used for validation of genotyping.

3) The availability of autopsy brain material from patients with MS and other neuroinflammatory CNS diseases, to validate identified genes by immunohistochemical/in situ hybridization localization and analysis.

4) The profound expertise in a wide array of in vitro and in vivo experimental models, including humanized animal models, for target validation and analysis of therapeutic compounds.

5) The availability of the technology necessary to generate new animal models (knock-in, knock-out, knock-down mutated mice) for target validation of candidate genes and evaluation of the efficacy of therapeutic compounds.

6) The availability of the technology necessary for high throughput analyses of gene and protein expression and further development of genomics tools for disease fingerprinting and evaluation of therapeutic efficacy.

7) The exploitation of different animal models to dissect the early events in neuronal degeneration which may lead to the identification of new therapeutic targets and biomarkers for evaluating the pathogenic process in MS and other neuroinflammatory diseases

8) The availability of a wide array of tools and products generated by the consortium participants to be tested for their anti-inflammatory and/or neuroprotective action. In this regard, the partners of the NeuroproMiSe consortium already own the intellectual properties of the products that will be used or developed in the project.

9) The strategy to focus on disease regulatory pathways identified by genetic analysis to develop new treatments for neuroinflammatory diseases.

10) The strategy to generate bifunctional molecules for targeting of neuroprotective activities to neurons as an innovative approach for the treatment of acute and chronic neuroinflammatory diseases.

11) The special attention to promotion and identification of intellectual properties and exploitable innovative aspects to increase the portfolio of the consortium and the industrial exploitation of NeuroproMiSe products.

12) The organization of integration activities, including training, exchange of personnel, set up of Reference Centers for Neuropathology, Genomics and Proteomics that will facilitate scientific and technical cooperation and optimise/speed up the execution of specific tasks.

The following results are expected within 5 years:

- Identification of new genes involved in MS and inflammatory neurodegeneration.
- Validation of the identified genes in human pathological material and animal models of immune-mediated glial and neuronal damage.
- Improved understanding of immunopathological processes involved in MS.
- Development of new animal models to study MS and other neuroinflammatory diseases.
- Development of new therapeutic compounds with anti-inflammatory and neuroprotective activities.
- Evaluation of the developed therapeutics in preclinical models of MS.
- Transfer of the developed therapies into other models of neuroinflammatory degeneration and evaluation of their neuroprotective efficacy.

Knowledge generated within the consortium will be protected by patents and disseminated subsequent to evaluation of proprietary contents by publications in relevant, peer-reviewed and highly recognized journals, by participation and presentation of the results at international meetings and by organization of neuroscience, immunology and medical oriented symposia within the consortium itself. All instruments of scientific communication (including the creation of a web-site for intra and extra-consortium communication) will be used to regularly follow the scientific and medical progress in the field, to adapt strategies and recruit new partners, if necessary.

Perspectively, NeuroproMiSe could also have major economic impacts by reducing massive health care, disability and social costs and by promoting biotechnology and pharmaceutical industry.

5.1 CONTRIBUTIONS TO STANDARDS

i) MS is a disease with profound inter-individual heterogeneity in clinical course and in the immunopathological mechanisms underlying the formation of brain lesions. Defining the genotype in relation to the clinical and pathological phenotype will significantly contribute to standards in diagnostics and genetic counseling. It is expected that specific genotypes will be associated with defined immunopathological mechanisms of lesion formation or with differences in the susceptibility of the target tissue to immune-mediated injury. When this is established, genotyping of MS patients may have a major influence in determining the disease prognosis of individual patients and in the design of therapeutic strategies that target specific mechanisms of demyelination and axonal injury in individual patients.

ii) All gene expression data attained in the project will meet strict criteria of gene expression analysis (according to MIAME – minimal information about a microarray experiment – Brazma et al., Nature Genetics (2001) 29(4): 365-371) and be made publically available by deposition in public data bases such as the EBI Database “ArrayExpress”.

5.2 CONTRIBUTION TO POLICY DEVELOPMENTS

The mission of NeuroproMiSe is to promote the scientific progress within the European Community and improve its technological competitiveness by performing a high quality multidisciplinary research based on the application of the most advanced biotechnologies and ranging from basic to preclinical studies. In terms of EC-policy related issues, the project aims to combine the clinical, scientific and manufacturing expertise of EU academic, governmental and industrial partners in a highly focussed effort to develop an effective cure for patients with inflammatory neurogenerative diseases, and hence to improve health and societal problems. Improvement and harmonization of protocols and procedures (e.g., tissue banks, animal models, high throughput genomic and proteomic analysis) will contribute to the establishment of common regulations to achieve and spread the highest research standards and generate validated tools to be widely used for preclinical studies of drug efficacy assessment. Establishment of new positions, training of scientists and mobilization of personnel across Europe will promote exchange of knowledge, technology and culture. The project not only aims to generate new knowledge and research tools but also to translate it into new products. One of the major aims of the project is to develop commercially exploitable products and hence to promote industrial production. In this respect, the industrial partners will provide the consortium with an adequate feedback in terms of general rules to be followed to promote the commercial development of any emerging product. To facilitate the commercial development of new products generated within the consortium, all European regulatory issues concerning approval for human use will be strictly followed. Patent applications will be filed to warrant the commercial exploitation of the deliverables. The Consortium Agreement will regulate all activities related to intellectual property rights, knowledge use, dissemination and exploitation.
5.3 RISK ASSESSMENT AND RELATED COMMUNICATION STRATEGIES

Not applicable
6. OUTLINE IMPLEMENTATION PLAN FOR THE WHOLE DURATION OF THE PROJECT

The overall aim of the NeuroproMiSe project is to carry out a programme of research at the forefront of the field of CNS inflammatory diseases, particularly MS. NeuroproMiSe will integrate basic research on immune and neurodegenerative/neuroprotective signalling pathways with proprietary technologies and reagents of industrial partners (including 3 SMEs) into an interdisciplinary translational research project leading to the generation of novel therapeutic interventions to counteract inflammation-mediated CNS tissue damage.

One of the major strengths of the NeuroproMiSe project is the availability of a wide array of technologies and pre-existing tools, animal models and *in vitro* systems, and already collected, relevant human disease material (mainly from MS patients) for genetic and neuropathological analysis. This array of expertise and materials coupled with the technological competence to generate new models and therapeutic compounds (in alliance with industrial partners), and with a deep knowledge of neuropathological, immunological and neurobiological issues related to MS and other neurodegenerative diseases, present a very good case for success in achieving the project aims. These will be pursued through the implementation of four scientific “Subprojects” oriented towards: identification of new susceptibility genes and target molecules using genetic/genomic/proteomic approaches (*Subproject Identification*), analysis of immunopathogenetic mechanisms (*Subproject Mechanisms*), validation of therapeutic compounds in preclinical models (*Subproject Validation*), and technological integration (*Subproject Horizontal Integration*). All these Subprojects are profoundly and reciprocally interconnected so that results generated in one Subproject may be exploited directly in others. For example, newly identified genes can be validated in a wide array of already established and newly generated model systems available through the consortium, and their interaction with specific inflammatory/neurodegenerative pathways under investigation can be evaluated. The fact that most participants will contribute specific tasks to several Subprojects will ensure a tight conceptual and methodological linkage of the Subprojects at the scientific and technological level.

Dedicated structures for training (*Subproject Training*) and management (*Subproject Management*) will be established and will ensure a further level of co-ordination of the activities, allowing monitoring of the programme progress. The project as a whole aims to transfer basic knowledge into new concepts and products to be tested in preclinical models and, in prospect, into pharmaceutical products and medical practice.

6A. ACTIVITIES

6.1 RESEARCH, TECHNOLOGICAL DEVELOPMENT AND INNOVATION ACTIVITIES

The major tasks of the project are:

1. Identification of disease-associated genes in EAE and experimental neurodegeneration.
2. Identification of disease-associated genes in MS case cohorts and families, exploiting the knowledge derived in experimental disease models.
3. Application of advanced genomics and proteomics to animal models of neuroinflammation and neurodegeneration (including myelin mutant mice, TREM2/DAP12 mutant/deficient mice, Wallerian neurodegeneration, MOG-EAE and TNF transgenic mice) to identify new targets for neuron- and axon- protective therapy as well as biomarkers of early and late signs of neuronal loss.
4. Validation of target genes identified in genetic studies and in studies of global transcriptome and proteome analysis using a wide array of already-existing and to-be-developed genetically modified mice (knockin, knockout and knockdown).
5. Deeper knowledge of the molecular mechanisms involved in innate (microglia) and adaptive (T-cell, B-cell) immune-mediated CNS tissue injury.
6. Development of new therapeutics based on disease-related genes identified and validated as above.
7. Testing of candidate small molecule and protein therapeutics developed according to the newly identified target genes for neuron- and axon-protective therapy.
8. Testing of new protein and peptide therapeutics for modulation of TREM-2 receptor function and signaling.
9. Design and evaluation of new therapeutics targeting the redox system (Ncf1 pathway).
10. Preclinical evaluation of TNF receptor subtype- and TNF intracellular signalling-specific protein and peptide therapeutics for neuroprotective therapy.
11. Evaluating the efficiency of novel ion channel blocking agents and glutamate receptor antagonists for neuroprotective therapy.

Subproject Identification: ‘Identification of candidate genes involved in inflammatory neurodegeneration’
Co-ordinator: P3
Co-coordinator: P8
Participants: P2b, P4, P5, P6, P7, P9, P10, P17, P19

Identification of genes related to inflammatory neurodegenerative diseases will be pursued by an innovative, comparative genetic approach in congeneric and intercrossed mice and rats, and well-characterized, ethnically homogeneous cohorts of MS patients and matched controls [Workpackages (WP) I1 to I3]. Besides genetic analyses, unbiased genomic and proteomic screens will be performed in different experimental models of neuroinflammation and neurodegeneration to pinpoint early events in neuronal degeneration (Workpackages I4 to I6). These strategies will lead to the identification of disease-associated genes and molecular pathways that will require further validation in appropriate model systems. These represent fundamental steps in the development of validated targets for new anti-inflammatory/neuroprotective therapies and the identification of novel biomarkers to monitor pathogenic processes and response to therapeutics.

i) Identification of polymorphic disease-regulating genes in mouse and rat platforms and confirmation in human MS

WP I1 and WP I2: Genetic studies in mice and rats
Within WPs I1 and I2, a ‘disease-driven’ strategy will be used to define genes associated with disease susceptibility and course in animal models of MS (both WPs) and neurodegeneration (WP I2). A single gene, small sets of genes, or genes with the same mechanistic pathways, defined in experimental animals will then be studied in sufficiently large and homogeneous cohorts of patients with MS and population-based controls.

Animal platform
P3 and P8 have since several years built an animal (mouse and rat) model platform suitable for identifying the relevant genes for inflammatory autoimmune diseases. This is based on the identification of the major loci controlling development of well-defined models of rheumatoid arthritis and MS. All these loci have been isolated in the so-called congeneric strains, and partial advanced intercross lines (PAIL) are already available. All the congeneric fragments have been placed in the same genetic backgrounds: C57BL/10 in the mouse and DA in the rat. This represents a major advantage as it allows comparison of the data and direct studies on gene interactions. In addition, P3 has selected...
and generated several new transgenic, mutated, knock-out and knock-in mouse lines, that are all bred into the same genetic background. This gives an advantage in the analysis of the pathways caused by the identified genes. Taken together, the animal platform is internationally unique and consists of 15000 mice and rats and over 200 unique strains.

Identification of genes controlling EAE and neurodegeneration

The strategy to isolate the genes will follow a well-defined scheme which has proven successful in the identification of two genes, \textit{Ncf-1} (Hultqvist et al., 2004; PNAS USA 101:12646) and \textit{CIITA} (Lidman et al., J Neurosci 2003; 23: 9817) controlling EAE and neurodegeneration, respectively.

i) The congenic strain will be tested for a series of disease and subphenotype traits, like clinical subtypes, demyelination, neurodegeneration, immune and inflammatory responses. For these studies, the following disease models will be utilized: mouse EAE models induced with a peptide of myelin basic protein (Jansson et al., 1991; Eur J Immunol, 21:693), whole spinal cord and myelin oligodendrocyte glycoprotein (MOG) (Weissert et al., J Clin Invest 1998; 102:1265); rat EAE models induced with MOG [all these models develop a chronic-relapsing disease course with MS-like demyelination (Storch et al, 1998; Brain Pathol. 8: 681) and axonal damage (Kornek et al, Am J Pathol 2000; 157: 267)]; a rat model of ventral nerve root avulsion-induced neurodegeneration and activation of CNS innate immunity (Lidman et al., J.Neurosci. 2003; 23:9817).

ii) The congenic fragment will be narrowed down, in order to localise the responsible gene. This will be done by search for recombination events in the congenic fragment and testing of each new congenic strain. Recombinants will be identified from large intercross breeding and screening of neonates or, alternatively, by screening of advanced intercross populations already established in P3 and P8 laboratories.

iii) Candidate genes will be selected in the minimal congenic fragments from bioinformatic analysis and sequencing of the actual strains. Both public databases and Celera are used as sources for the bioinformatic analysis. Expression analysis will be also used, based on both RNA microarrays and proteomic analysis to obtain information on the relevant candidate genes.

iv) Candidate genes will be validated using functional and transgenic approaches (see below).

Cluster analysis and epistasis

A critical value of the animal model platform for understanding complex diseases, including MS, is the possibility to identify genetic interactions. This concerns both interactions occurring between linked genes (selected together as a cluster), and unlinked genes. These interactions will be determined through analysis of large segregating crosses using relevant software like the R program and utilizing the database introduced by P17. Once these interactions have been predicted, it will be possible to actually prove them by crossbreeding the relevant congenic strains and generating partial advanced intercross lines (PAIL). In PAIL, a large number of recombinant fragments is collected, which increases the power of the analysis both between congenic fragments on different chromosomes as well as fragments within the same chromosome.

An example of this approach stems from previous attempts to find the genes in the first gene regions outside MHC identified for autoimmune diseases, eae2 and eae3 (Sundvall et al., 1995; Nature Genet. 10:313). One of these regions (eae3), located on chromosome 3, controls both EAE and collagen-induced arthritis (CIA) and seems to contain several genes regulating different parts of the disease. In addition, the eae3 locus interacts with a locus on chromosome 15 (eae2). Most likely, this represents a typical cluster of genes that have been selected together and also operate through inter-chromosomal interactions. To further dissect and understand these interactions, a PAIL of chr3 and chr15 congenics has been generated and a population of mice with large numbers of recombinations in the relevant loci is available in which the various genes and their interactions can be identified. So far, 3 loci on chr3 and 2 loci on chr15 have been identified, several of which interact with each other.

Finally, the real breakthrough in identification of epistasis is after cloning of some of the genes. This will allow fixation of one of the interacting genes and thereby increasing the power of the analysis of both congenic crossbreeding and analysis of the advanced intercross population. One example is the recently identified \textit{Ncf1} gene. The E3 allele dominantly suppresses the disease in the DA background, but the reverse is not true in the E3 background. Calculations of genetically segregated populations
suggest several interacting genes that can now be specifically investigated, some of which require the establishment of new congenics.

**Target validation**

Once the genes and gene clusters are identified, a hypothesis-driven approach will be pursued to validate their role and dissect the pathogenic pathways. This will include a wide set of functional tests performed in many laboratories within the consortium. The NeuroproMiSe project puts emphasis on this activity, using a large variety of available tools, as well as emerging technologies towards this aim. Three of the participating groups (P3, P8 and P9) are European leaders in this field, and have developed sets of complementary tools that allow detailed functional studies, leading to the understanding of the mechanisms by which genes are involved in the disease or related phenotypes, and defining biochemical pathways. The aim is to validate the genes identified through the genetic approach, define the functional effect of genetic variants in these genes, and understand the mechanisms by which they contribute to the disease risk, as well as describe the biochemical and physiological pathways involved and their relation with the disease condition. The studies in animal models will provide new specific functional hypotheses to test in human neuroimmune disease (see below).

Several methods are available for functional studies of genes in animal models, which will be used singly or jointly. These allow for a very wide range of explorations, which will be done in parallel with the candidate gene sequencing to identify genetic variants between strains with differential phenotypes: a) gene expression analysis will be performed using both full genome chips (for pathway analysis) and specifically designed QTL chips (for candidate gene identification); b) differential protein analysis on 2D gels followed by mass spectrometric analysis; c) lastly, the identified candidate genes will be confirmed conclusively through functional testing using either the minimal congenic fragment or specifically designed mouse strains. These strains will be created through lentivirus-based genetic manipulations and ES-cell based knock-in approaches to insert the relevant mutation, or gene targeting using RNAi technology. The creation of bone marrow chimeras will permit to identify the contribution of genes in different cell populations and provide a vehicle for retroviral transfection in gene complementation studies. Further fine dissection will be obtained in immunoglobulin and T-cell receptor transgenic models and in humanized transgenics which will be generated in collaboration with P4 and P6, enabling the creation of systems for refined functional tests, getting closer to the human situation. Double transgenics, or combinations of constructs, will allow further testing for specific gene interactions.

**Bioinformatic integration.**

Expression studies, proteomics and structural analyses performed in animal models and in human systems will be integrated in cooperative studies addressing specific genes, using a data-base developed by P17. This is a genotype-phenotype relation database open to the consortium (see Subproject Horizontal Integration).

**WP I3: Genetic studies in MS patients**

The HLA-DR,DQ haplotype DR15,DQ6 is the major genetic risk factor in MS. Members of P8 team have identified a second risk gene in the HLA region, the HLA class I allele HLA-A*0201 in the Swedish population (Fogdell-Hahn et al. 2000; Tissue Antigens 55:140). Recently, this finding has been corroborated in an extended study of over 750 MS patients and 750 controls, showing a decreased risk conferred by the HLA-A*0201 allele (OR=0.5, P=10^{-12}) (Hillert et al., unpublished data). DR15,DQ6 and A*0201, located 2.6 Mb apart, have independent effects but interact to affect the risk of MS.

In spite of the difficulties in MS genetics outlined above, P8 and P9 teams have recently been successful in finding non-HLA MS genes. By dissecting a suggested linkage peak identified in a previous genome screen in Finnish MS families (Kuokkanen et al., 1997; Am J Hum Genet. 61:1379), P9 has identified the PRKCA gene in 17q as a risk gene for MS (Saarela et al., 2002; Hum Mol Genet. 11:2257). The P9 team has also identified an associated locus on 5p in the region syntenic with a rodent Eae 2 locus. Along with others, the P8 team has reported an influence of CTLA-4 for the risk of
MS (Ligers et al. 1999; J Neuroimmunol. 97:182), as well as for the course of MS in the Swedish population (Ligers et al. 2001; Genes Immun. 2:145). Thus, it seems that the genetic background among Finnish and Swedish MS patients is sufficiently restricted to allow the initial gene identification. Within this project, it is proposed to expand the study samples in these two populations and confirm the established findings across the Nordic study samples. This effort should help to confirm the role of specific genes in the molecular pathogenesis of MS. These study samples with well defined phenotypes will be of extreme value when the impact of identified rodent genes is tested for human MS.

Members of P3, P8 and P9 teams have already pioneered the field of comparative genomics in MS, supporting the validity of this approach. In 1996, P3 and P9 (Kuokkanen et al. 1996; Nature Genet. 13:477) discovered an MS linkage peak syntenic to a mouse EAE QTL. Similarly, P8 identified MS linkage peaks corresponding to rat EAE loci (Xu et al. 2001; Eur J Hum Genet.9:458). More recently, P8 has identified a role for the \textit{CIITA} gene (a gene regulating HLA gene expression) in MS and in other inflammatory diseases, by first identifying the gene in an animal model and by extending the analysis to the human gene (Swanberg et al. 2005, Nature Genet., 37:486). Thus, the strategy proposed has already led to the identification of disease-relevant genes. This new situation offers a new level of complexity but also new opportunities. A prerequisite will be the assessment of new genotypic information in the context of already known genes to control for epistasis and other gene-gene interactions. This needs to be performed in well-characterised and ethnically homogeneous populations. The proposed study will take advantage of the special character of two unique MS populations, the genetically original and relatively isolated Finnish MS population including many MS families, and the high prevalence Stockholm-region MS population, representing the supposed Scandinavian founder population for MS in Europeans (“the Viking hypothesis”).

Participants P8 and P9 will establish a resource that enables a rapid testing of new candidate genes, identified primarily via comparative genomics, with high density SNP markers in sufficiently large data-sets of sporadic as well as familial MS, and allows a streamlined assessment of disease, risk, severity and disease course as well as gene-gene interaction. The data-set will be unique from an ethnic point of view, as it offers a documented ethnic homogeneity with full background genotyping data for already identified MS genes, allowing gene-gene interaction analysis with the potential of gene-environment analysis. The resource will consist of over 4,000 individuals including 1,500 unrelated Swedish MS patients and ethnically matched controls, 40 extended MS families from Finland and over 700 Finnish MS trios, each individual clinically characterised. High-throughput SNP genotyping with densely spaced markers (1 per 1-2 kilo base pairs) will be performed for selected genes.

Within WP I3, SNP analysis of selected genes will be also performed in a smaller series of MS patients (about 100) for which neuropathological information is available. Brain biopsy material has been collected by participant P2b and DNA extracted from the blood of the same patients is also available. This material will allow to correlate the genetic information with the identified patterns of tissue damage. At a later time, SNP analyses will be extended to a larger series of autopsy brain material available from P2b and P5.

\textbf{ii) Global gene transcription and protein expression profiling in experimental models of neurodegeneration}

Within the Subproject Identification, three distinct animal models of neuronal degeneration will be examined to characterize nervous tissue and cell specific changes in gene transcription and protein expression in the absence of overt inflammatory reactions (WPs I4-I6). Such an approach will help to pinpoint early changes in neuronal damage and associated glial reactions and to determine whether or not common pathways of axonal and neuronal damage are triggered in different model systems and might be targeted by therapeutic interventions. Participants P7, P10 and P19, leading WPs I4, I5, and I6, respectively, have long-standing experience in the analysis of axonal/neuronal and myelin degeneration in different experimental models and all of them have provided important contributions in this field.

WP I4 aims to analyze gene expression changes accompanying neurodegeneration in mice lacking myelin-specific genes. Mice with deletions in 2',3'-cyclic nucleotide phosphodiesterase (\textit{CNPI}) and
proteolipid protein (*PLP1*) genes have been generated by P7, which undergo progressive, severe neurodegenerative changes, including axonal swellings and loss, although in the presence of nearly normal CNS myelin. The onset and severity of the axonal phenotypes is highly reproducible and allows identification of the early neuronal changes that precede axonal loss. These well characterized mice will be used to identify the molecular pathways and monitor altered gene expression that is associated and possibly critical for the axonal damage. Different ‘myelin’ mutant mice with similar axonal and neurological phenotypes will be analyzed to cross-control the microarray results, and to cross-validate potential therapeutic interventions. A major innovative aspect of the study performed in WP I4 is that it allows, after transgenic labelling of cell nuclei, the isolation of defined neuronal and oligodendrocyte populations from mouse brain and cerebellar sections by fluorescence-directed laser microdissection. Gene chip microarray analysis will be performed using as few as 50 cells. This strategy will thus enable, for the first time, a cell specific and temporal analysis of the molecular changes in distinct glial and neuronal cell populations during neurodegeneration and could be successfully applied to other disease models.

WP I5 aims to analyze changes in gene transcription associated with neuronal dysfunction and neurodegeneration in TREM-2/DAP12 mutant/deficient mice. Mice with knock down of the triggering receptor expressed on myeloid cells-2 (TREM-2) (generated by P19) as well as DAP12 mutant mice (available by P9) will be analyzed at distinct stages of disease development to identify new targets of inflammatory neurodegeneration. cDNA microarray technology will be used to study gene expression modifications in different strains of DAP12 mutants, TREM2 mutants and TREM2 knock-down mice. The results will be inter-crossed to identify new targets for therapeutic intervention. TREM-2, a member of the innate immune receptor TREM-family, is expressed on the cell membrane of monocyte-derived dendritic cells, osteoclasts and microglia. It is associated with the signaling molecule DAP12 and it was suggested that an unknown TREM2 ligand results in the phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasmic domain of DAP12. Recently, mutations in TREM-2 and DAP12 have been described by P9 in polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL)/Nasu-Hakola disease patients. Both synaptic dysfunction and synaptic loss appear to be associated with the early stages of disease development, possibly being responsible for the early psychiatry symptoms of the patients. At later disease stages, the patients show myelin loss and neurodegeneration leading to dementia and premature death. Deficiency in DAP12/TREM-2 also results in bone abnormalities due to impaired osteoclast differentiation and function. The mechanism underlying neurodegeneration remains unclear. Thus, mice with altered TREM-2/DAP12 signaling should offer the advantage of analysing synaptic dysfunction and neuronal degeneration as a result of microglial dysregulation. Because overt inflammatory processes are detrimental to the brain tissue and CNS innate immunity has to be strictly controlled, the study of TREM-2 activated pathways will complement the investigations carried out in the Subproject Validation, in which TREM-2 agonists and antagonists will be evaluated for their immunomodulatory activities in *in vivo* and *in vitro* models (WP V1, see below).

WP I6 aims to analyze protein changes accompanying Wallerian degeneration. Injury to an axon or the neuronal cell body results in degeneration of the axon distal to the lesion. This form of degeneration is known as Wallerian degeneration and accompanies diverse injuries to the CNS including immune-mediated injury in MS, ischemic and traumatic injury. The mechanisms that underlie axon degeneration are poorly understood but it appears that axons degenerate by an active process akin to programmed-cell-death. As the axon undergoes Wallerian degeneration the response of macrophages and microglia will be influenced by the mode of degeneration. Within WP I6, a mouse model of axon degeneration, transection of the optic nerve, will be establish to analyze changes in global protein expression at different times after the insult. Proteomic analysis will be performed in collaboration with the Centre for Proteomics of the University of Southampton (P10) and will make use of Isotype Coded Affinity Tag (ICAT) which allows a quantitative estimate of protein changes and detection of post-translational modifications. These studies investigating changes in protein expression in degenerating fibre tracts will directly complement the genomic studies to be carried out in the animal models described above. The model of optic nerve transection will be also exploited to examine how systemic inflammation triggered by an infectious agent may affect neuronal degeneration. This is a critical issue to investigate, since it is well known that MS relapses are frequently associated with infectious episodes, suggesting that activation of the immune system might have an impact on ongoing neuronal degeneration.
In summary, these components of the Subproject Identification will determine changes in gene transcription and protein expression during neuronal/axonal degeneration at early and advanced stages. The results obtained in the different models will be compared in the search for disease-associated and disease-specific patterns in the expression of single genes/proteins or molecular pathways. These studies will also underpin subsequent studies in more complex model systems such as EAE, in collaboration with other participants (P1, P2a, P3, P4, P6, P8). The differential expression of candidate genes/proteins will be confirmed by independent techniques including quantitative RT-PCR, in situ hybridisation and immunohistochemistry (if antibodies are available), in collaboration with P2b and P5. The functional verification of the most promising gene/protein candidates will be achieved through the generation of new transgenic, gene knockdown, knockin or knockout mice, as appropriate, using the expertise of several participant laboratories in conventional and conditional gene targeting technologies in mice. It is expected that candidate neuroprotective molecules will be identified or developed by the appropriate industrial partners for therapeutic applications.

Subproject Mechanisms: ‘Analysis of key effector mechanisms of inflammatory CNS injury’

Coordinator: P1
Co-coordinator: P6

Participants: P2a, P2b, P3, P4, P5, P10, P19

The major task of this subproject is to elucidate the mechanisms underlying immune-mediated CNS tissue damage and to identify target molecules/pathways suitable for therapeutic intervention. The focus will be on specific pathways that appear to be directly involved in demyelinating/neurodegenerative processes in MS according to previous studies performed in the participants’ laboratories. The involvement in CNS immunopathological processes of candidate genes and pathways identified by genetic and expression profiling analyses (Subproject Identification) will be also investigated.

The Subproject ‘Mechanisms’ is composed of four WPs (M1 to M4) and focuses on major putative components of immune-mediated CNS damage: CD4+ and CD8+ T-cell autoimmunity, abnormal lymphoid neogenesis in the inflamed CNS and microglia/macrophage-mediated axonal/neuronal injury.

**WP M1** will specifically focus on CD8+ T cell-driven neurotoxicity and related neuroprotective strategies and will involve several participants (P2a, P5, P6). To further characterize the role of autoreactive CD8+ T cells in CNS tissue damage in vivo and to test new therapeutic strategies aiming at CNS tissue preservation, a hemagglutinin (HA) transgenic mouse model of CD8+ T-mediated neuroinflammation generated by participant P6 will be used. In this transgenic system, expression of HA in neurons or glial cells is either constitutive, inducible or doxycycline-controlled and enables the targeting of the immune response toward specific CNS cell types. The neuroprotective strategies will aim at either inhibiting the activation, inhibiting the migration, or blocking the effector molecules of pathogenic CD8+ T cells (P6) or at protecting/repairing the CNS tissue (P2a). The doxycycline-controlled HA transgenic model has the advantage that it enables testing of therapeutic approaches both during CNS inflammation and after inflammation is shut down. Studies to be performed in WP M1 will also address the pathogenic properties of autoreactive CD8+ T cells recognizing a HLA:neural self-peptide complex relevant to the human disease. This will be examined using already available humanized HLA-class I transgenic mouse models. In these mice, the human class I molecules, such as HLA-A2, are expressed ubiquitously whereas the murine class I genes have been deleted by homologous recombination, so that HLA-A2 molecules are their sole class I molecules expressed. Initial experiments will be devoted to establish the conditions to induce or potentiate CD8+ T cell-mediated autoimmune encephalomyelitis using immunogenic HLA-A201-binding neural self-peptides in both active and passive immunization protocols. In a second step, myelin-specific CD8+ T cells will be isolated from HLA-A2 positive MS patients using HLA-A2:peptide tetramers, their T cell receptor will be cloned and T-cell receptor transgenic mice will be generated. To address the pathogenic relevance of MS patient-derived autoreactive CD8+ T cells in vivo, the TCR transgenic mice will be crossed with the HLA-A2 humanized mice and CNS autoimmune disease induction will be evaluated in the resulting double transgenic mice. The specific relevance of this model is that it...
should accelerate the translation of the pathophysiologic and therapeutic information gained from the study of animal models to the human situation, taking advantage of the humanized nature of the transgenic model.

**WP M2** will address the disease modifying effect of epistatic interactions in MS-associated MHC class I and class II molecules and will involve collaborative interactions between participants P3, P4, P5 and P6. Preliminary studies performed by participant P4 have shown how two MS-associated MHC class II molecules (DR15 and DR51) can interact in a way that leads to a disease course in a humanized mouse that closely resembles MS, being associated with both CNS inflammation and demyelination. Within WP M2, the cellular, molecular, biophysical and structural bases for this disease modifying interaction will be investigated by generation of mice that express cross-reactive T cell receptors from MS patients. These T-cell receptors can recognize different MHC class II molecules presenting different peptides derived from autoantigens and microorganisms such as virus and bacteria, and this cross-reactivity has important clinical implications with regard to disease course and disease manifestations (Lang et al., Nat. Immunol. 2002;3:940). Following immunization with different autoantigens or infection with viruses/bacteria that contain known molecular mimicry T-cell epitopes, the clinical and neuropathological consequences of T-cell receptor cross-reactivity as well as cytokine secretion and signalling pathways in T cells from these humanized transgenic mice will be investigated. Soluble T-cell receptors and soluble MHC molecules will be produced and used to measure their affinity for each other by using Biacore analysis and to obtain high resolution three dimensional atomic level insight into their interaction by using x-ray crystallography (in collaboration with Prof Y. Jones, Oxford). This information will be important for the understanding of how disease pathways can be modified, and ultimately, therefore also, for the design of new drugs.

Recent genetic studies have pointed to a modifying role of certain MHC class I alleles, in particular HLA-A2, in MS. In contrast, HLA-A3 has been shown to act as an MHC class II independent risk factor. To investigate the contrasting effects on MS of these two HLA class I molecules transgenic mice expressing HLA-A2 and HLA-A3 will be crossed with the already existing humanized mice that develop MS-like disease (described above). If an A2-associated disease modifying effect or an A3-associated disease-contributing effect is observed in the humanized mice, the mechanisms behind these effects will be further analyzed. The ramifications of this research may lead to a broader understanding of the role of adaptive immunity in MS, and accordingly also to a new rational way of developing disease modifying drugs.

**WP M3** aims to clarify the contribution of EAE- and MS-associated abnormal lymphoid neogenesis (ectopic B-cell follicles identified by P1, and so far described to be confined to the inflamed meninges) to disease pathogenesis and neurodegenerative changes, and to exploit this information to develop novel therapeutic concepts (e.g. complementing systemic disease-modifying treatments with intrathecal delivery of specific immunomodulatory compounds) and strategies to intercept the pathways regulating intracerebral lymphoid neogenesis. As a first step, a detailed immunohistochemical analysis of post-mortem MS brain tissue will be performed by P1 to establish whether and to which extent the presence of intrameningeal lymphoid follicles associates with lesional activity and degree of demyelination/neurodegeneration. Following selection of MS tissue samples containing clearly identifiable ectopic B-cell follicles, laser microdissection of selected CNS compartments (intrameningeal lymphoid follicles, intraparenchymal and subpial demyelinating lesions, and normal appearing white matter) and gene expression analysis (gene microarrays and quantitative RT-PCR, depending on the availability and quality of snap frozen brain tissue) will be carried out to identify genes and molecular pathways involved in lymphoid neogenesis and associated tissue damage. In parallel, studies performed in appropriate mouse strains will aim to understand whether CNS overexpression of molecules regulating lymphoid neogenesis (like homeostatic lymphoid chemokines and B-cell survival factors) might induce/enhance intrathecal formation of ectopic B-cell follicles. This will be achieved through the construction of lentiviral vectors expressing genes encoding murine CXCL13 and BAFF and their injection into the ventricular/meningeal compartment of the mouse CNS. Following assessment of transgene expression and induction of EAE, clinical, immunological and neuropathological parameters of disease evolution will be analysed (in collaboration with P2b, P5 and P6). The generation of animal models reproducing features of intrathecal humoral immunity in MS patients will represent a considerable advantage for studies evaluating the efficacy of potentially therapeutic compounds. A subsequent step will be to evaluate the
therapeutic effect in EAE models of intracerebrally injected compounds that block the activity of molecules promoting lymphoid tissue formation and B-cell immunity (e.g. decoy receptors, such as the fusion protein BAFFR-IgG, or neutralizing anti-CXCL13 antibodies). The ultimate goal of WP M3 is to find out whether preventing the establishment of an intracerebral lymphoid environment might stop or reduce CNS inflammation and neurodegenerative processes.

WP M4 will investigate the involvement of microglia/macrophages in axonal/neuronal injury. P19 will use in vitro models of neuronal microglial co-culture or organotypic brain tissue cultures to study the role of microglial or macrophage derived molecules in axonal/ neuronal injury. Particularly, macrophage/microglial derived mediators will be identified, which are involved in axonal/neuronal injury. Soluble mediators will be blocked by neutralizing antibodies. Macrophage/microglial genes will be specifically knocked-down using short-hairpin RNA interference of lentiviral vectors. Injury to axons/neurons will be monitored by high resolution confocal microscopy imaging after fixation. Furthermore, neurons will be transfected with GFP-tagged axonal and synaptic molecules to visualize axonal/neuronal dysfunction and early signs of injury after challenge with microglia/ macrophages. Identified protective compounds will be tested in this microglia-neuron culture model. Furthermore, newly identified candidate genes will be overexpressed in neurons to protect axons against inflammatory injury. In a second step, in vivo models (transgenic mice generated by lentiviral vectors) of microglia/macrophage mediated axonal injury will be established. Therefore, lentiviral vectors will be used for transduction of fertilized oocytes and generation of transgenic mice. Mechanisms and signalling pathways of axonal injury will be analyzed in these in vivo models.

In summary, the activities performed within the Subproject Mechanisms will increase our knowledge of immunopathological mechanisms underlying CNS neuroinflammation, will contribute to the identification of new targets for neuroprotective interventions, and will generate animal models useful for evaluating the therapeutic efficacy of compounds identified in other subprojects.

Subproject Validation: Development of new therapeutics for neuroprotection based on targets identified and validated in animal models

Coordinator: P19
Co-coordinator: P11
Participants: P1, P2a, P2b, P3, P5, P6, P12, P13, P14, P16, P17, P18, P20

The aim of the ‘Subproject Validation’ is to develop and validate new therapeutics based on targets identified in previous work by the participants (P2a, P3, P11, P12, P13, P14, P15 and P17), and at a later stage on new targets identified and validated through the activities of the Subprojects ‘Identification’ and ‘Mechanisms’. Four biotechnology companies (3 of which are SMEs) closely interacting with academic/governmental teams contribute to this Subproject. The availability within the consortium of a number of promising therapeutics and of a wide array of in vitro, semi-in vivo and in vivo model systems to test their activities represents a great advantage, as it will allow the comparison of the neuroprotective efficacy and the evaluation of different combination therapies in various experimental settings.

WP V1 aims to validate the role of TREM-2 in CNS inflammation and to determine the efficacy of compounds modulating TREM-2 activity in achieving neuroprotection in animal models of MS. TREM-2 is an immunoglobulin-like cell surface receptor associated with DAP12 that activates dendritic cells in vitro. Work by P9 group has recently shown that genetic defects of human DAP12 and TREM-2 result in a rare syndrome characterized by bone cysts and presenile dementia called Nasu-Hakola disease (Paloneva et al., Nature Genet. 2000; 25:357). This observation suggests that TREM-2 may function in myeloid cells other than dendritic cells, including macrophages/microglia. Indeed, recent work by P19 indicates that TREM-2 modulates microglia activities, including phagocytosis and production of inflammatory mediators (Takahashi et al., J Exp Med. 2005; 201:647).

WP V1 is coordinated by P19 with the contribution of P1. It aims to evaluate the therapeutic efficacy of a number of products provided by an external company (Bioxell, Milan Italy) and capable of modulating (either mimicking or blocking) TREM-2 activity in different in vitro and in vivo model systems, and to get further insights into the role of TREM-2 in microglia biology, cell-to-cell
interactions and inflammation in the CNS.

WP V2 aims to validate the role of Ncf1 in inflammatory processes and to develop reagents affecting redox pathways that might be used for therapeutic purposes. Ncf1 encodes the activating component of the NADPH oxidase complex which is responsible for production of reactive oxygen species (ROS) and is expressed in myeloid cells, including macrophages/microglia, dendritic cells and neutrophils. Contrary to the dogma that ROS are detrimental in inflammatory disorders, preliminary evidence by P3 and P17 indicates that the oxidative burst effects controlled by Ncf1 play a critical role in reducing T-cell activation and development of the T-cell repertoire, suggesting that ROS-like peroxides could be exported into the synapse between T cells and antigen presenting cells (APCs), thus affecting T-cell activation. A specific agonist of the NADPH oxidase complex in cells that make contact with encephalitogenic T cells is therefore anticipated to decrease the autoimmune response and prevent the neurodegeneration caused by inflammation. This question will be addressed both in rat and mouse systems, in which unique tools in the form of animal models, congenic (rat) and mutated strains (mouse) have been developed. Studies to be performed in the rat will address the role of Ncf1 during T-cell selection in the thymus, physiological selection in the peripheral lymph nodes, immune priming in lymph nodes and effector activation in the target tissue. Both the interacting APC and the responding T cell will be isolated and, using a functional genomic approach (cDNA microarray, and 2D gel analysis and massspectrometry), all differentially expressed products, that differ between the wild type DA rat and the congenic Ncf1 mutated DA rat, will be investigated. The information will be analysed using bioinformatics and interesting candidates will be investigated further using appropriate phenotypic and functional methods. T cells and APCs isolated from mice carrying a Ncf1 mutation and backcrossed into an EAE-susceptible mouse strain will be also utilized to dissect the pathogenic role of Ncf1 in immune responses. Also in this case, a functional genomic analysis will be performed to delineate the Ncf1-associated pathway and hopefully reveal unique targets for new therapeutic approaches. The coordinator of this subproject, P17, is a biotechnology company with a primary interest in identifying compounds that selectively increase the activity of the NADPH oxidase. Recombinant cell systems and immortalised cell lines expressing a functional NADPH oxidase will be used to analyse the efficacy of new chemical structures and make comparisons with lead compounds. Selected compounds will be tested in mouse and rat models of EAE with different clinical courses.

WP V3-V4: Agents selectively targeting TNFR1 or TNFR2 receptor and signalling pathway are being developed and tested in the following two workpackages with the aim to block inflammation and selectively promote neuroprotective function.

WP V3 aims to achieve neuroprotection by selective stimulation of TNFR2. Genetic engineering combined with nanocarrier technology forms the basis to design new classes of TNF receptor selective reagents. Participant P12 has constructed a novel “single chain” TNF molecule (scTNF) and receptor-selective variants thereof, in which the three monomers are genetically linked by short Gly-Ser linkers to constitute a stable trimer with superior properties with respect to stability and covalent coupling to matrices. These constructs form the basis for fusion proteins containing an amino-terminal immobilization domain recognizing neuronal cells and, as carboxy-terminal domain, a TNFR2 selective TNF or scTNF mutant. TNFR2 selectivity is ensured by introducing mutations in the TNF molecule; local activation is reached by the specificity of the immobilization domain and the fact that only the cell surface immobilized TNF moiety will be bioactive. This principle will be applied for creation of TNFR2 specific TNF targeted to neuronal tissues. These constructs will be further developed and characterized with respect to their expression in appropriate hosts, binding properties, specificity, in-vitro and in-vivo stability and toxicity in collaboration with P20.

mAbs specific for the widely expressed neurotransmitter receptor subunit NMDAR1 (NR1) are available (P12, P13). mAbs will be identified that do neither interfere with nor activate on their own, glutamate responsiveness of NR1 and exploit their use as a targeting domain for TNFR specific reagents. TNF can also be targeted to oligodendrocytes, via e.g. mAbs recognizing cell-specific surface markers, like O4 or O1 (galactocerebroside). A second strategy makes use of a covalent, oriented coupling of TNF to nanoparticle carriers, thereby mimicking a membrane expressed TNF molecule (P12). Using this technology for a TNFR2 specific mutein, which has little bioactivity as a secreted trimeric molecule, a specifically TNFR2 activating nanoparticle will be created. The conceptual advantage of this approach is several fold: nanoparticles can be sequentially conjugated at the surface with additional reactants, e.g. to enhance BBB passage and/or targeting damaged brain
tissue; they can be used in parallel for encapsulation of other therapeutics; they differ in pharmacokinetic properties from soluble protein/peptide therapeutics and show a size dependent capability to enter perivascular space and intracellular uptake via an endocytosis/lysosomal dependent or independent pathway, offering alternative routes of drug delivery and/or signal propagation. Further development and optimization of coupling chemistry and particle properties will be performed by P12 in collaboration with P20.

As a complementary approach to modulate TNF signals, blockade of TNFR1 via receptor antagonists as an anti-inflammatory strategy will be pursued. Participant P12 has previously generated a human TNFR1 specific antagonist reagent as a potential therapeutic (US patent No. 5736138). In addition, murine TNFR specific antibodies suited for established murine EAE models will be also available through partner P16, allowing an immediate exploitation of the in vivo efficacy of these reagents. The expected function of the above mentioned designer molecules will be tested in a hierarchical order with increasing complexity of the models first in in vitro systems of established cell lines and then primary neuronal and oligodendrocyte cultures exposed to a toxic insult, in collaboration with other participants (P1, P13, P19). Successful candidate molecules will be produced in a preclinical scale (participant P16) and analyzed in EAE and ischemia disease models (P1, P3, P6, P13). To investigate the functionality of human specific protein therapeutics in vivo, humanized TNFR1 and TNFR2 mouse lines will be generated by knock-in strategies (P13). By crossbreeding, double TNFR exchange mice will be available (P13). Primary neuronal and oligodendroglial cell cultures prepared from these mice will also allow to investigate the functionality of human specific reagents in vitro.

WP V4 aims to identify and validate the key intracellular proteins that are responsible for a recently identified pathway of TNFR1 neuroprotection that operates to prevent delayed (apoptotic) neuron death, and to use this knowledge to rationally design cell-penetrating peptides that will enhance the function of this pathway independently of extracellular TNF ligand/receptor engagement. P14 will work to define the key signaling mediators of NFkB activation and neuroprotection in neurons, using techniques of gene knockdown (lentiviral delivery of siRNA) and protein inhibition (lentiviral delivery of dominant negative proteins), and to develop molecular forms with enhanced neuroprotective function (fusion of wild-type or mutant antiapoptotic proteins with the TAT sequence of HIV for efficient intracellular delivery). The efficiency of these reagents will be assessed in primary neurons (cortical, hippocampal) undergoing death after glucose deprivation, glucose/oxygen deprivation and kainic acid excitotoxicity. The most promising candidates will be tested in vivo using models of brain injury such as cerebral ischemia, kainic acid-induced epileptic seizures and MOG-EAE. Potential drug leads will be further developed into neuron-targeted drugs, using strategies similar to those described in WP V3, in later stages of the project. The goal is to preserve neuron integrity in acute CNS injuries but also in chronic immune-mediated pathologies that involve significant secondary neuron and axon damage such as MS. The ultimate aim will be to develop neuron-specific cytoprotective reagents that will be constitutively activated and function independently of disease- or drug-induced changes within the extracellular environment.

WP V5 aims to compare the efficacy in axonal protection of different types of sodium channel blocking agents and inhibitors of the reverse sodium-calcium exchanger. Collaborative agreements already exist with several European pharmacological companies to provide suitable agents for examination. The efficacy of the different drugs and different approaches will be tested by P11 using an already proven, stepwise screening progression through in vitro (Smith et al., Ann Neurol., 2001, 49:470-476), b) semi in vivo (Kapoor et al, Ann Neurol., 2003; 53: 174), and c) fully in vivo models (Bechtold et al., Ann Neurol, 2004; 55:607), in combination with electrophysiological, morphometric and behavioural techniques. Thus, the promising drugs identified in a) will be ranked for efficacy and examined in b), after which the best will be examined in c). The best drugs will be tested in vivo in animal models of MS, including MOG-induced EAE. The best drugs identified in EAE will be advanced for clinical trial in MS patients. The proposed methods and strategy are realistic, as proven by the successful identification of lamotrigine as an effective agent for axonal protection. This drug will be examined in a MS clinical trial funded by the British MS Society and GlaxoSmithKline. Apart from MS, it is reasonable to believe that the results will be relevant to a range of disorders in which inflammation and nitric oxide are expected to play an important role, including motor neuron disease, the neurological complications of AIDS, cerebral malaria, Guillain-Barré syndrome, and Parkinson’s disease.
WP V6 aims to evaluate the efficacy of calcium channel blocking agents and glutamate receptor antagonists in axonal protection. As a first step, it will be investigated how glutamate and calcium channel dysfunction cause neuronal/axonal damage in the inflamed EAE spinal cord in order to develop novel therapeutic approaches for neuroprotection in MS. This WP is going to involve a SME (ELL, P18) and P11, working in close collaboration in the vitro, semi-in vivo and in vivo models of axonal degeneration described in WP V5. ELL have a range of novel pharmacological agents to assist in this research, including glutamate receptor antagonists to reduce the risk of excitotoxicity, and glutamate uptake inhibitors to exacerbate and thereby illustrate the importance of glutamate-mediated damage. Blockers of sodium-calcium exchange and calcium channel function are also available to prevent calcium loading of axons. Calcium imaging techniques will be utilized in spinal cord slices from control and EAE-affected rats or mice to analyze spontaneous activity and intercellular communication in both neuronal and glial compartments.

WP V7 aims to identify anti-apoptotic neuroprotective strategies in EAE and to verify their efficacy in combination with established immunomodulatory and anti-inflammatory agents. These studies will be performed by P2a in a model of EAE induced by myelin oligodendrocyte glycoprotein (MOG) in Brown Norway rats which reproduces the neurodegenerative aspects of MS. Pathways involving the Bcl-2 family of proteins, mitogen-activated protein kinases, and the phosphatidylinositol-3-kinase/Akt pathway will be manipulated by treatment with neurotrophic factors, neurotrophin-like substances such as erythropoietin, pharmacological approaches, and protein transfer tools (TAT-fusion proteins). For the latter, TAT-proteins transducing GDNF or Bcl-2, an anti-apoptotic member of the Bcl-2 family, have been developed. In all the treatment groups, cytokine profiles and inflammatory infiltration within the optic nerves will be quantitatively and qualitatively assessed. To allow fast transfer of the results to clinical application, pharmacological agents will be used that have already been approved for the treatment of human subjects in other disease contexts or have been demonstrated to exert protective properties in “classical” neurodegenerative disease models. These agents include statins, a group of cholesterol-lowering agents, and minocyclin, a member of the group of antibiotics. Another promising candidate substance is flupirtine, an analgesic that has been shown to up-regulate Bcl-2. During an initial screening period, this wide spectrum of approaches will be tested only with respect to neuronal/axonal survival and functional (clinical and electrophysiological) effects. In a second step, therapy-related signal transduction of those agents which show neuroprotective effects will be investigated. In this context, studies will include detection of proteins such as BAG-1 or other Bcl-2 family members that are known to be involved in neurodegenerative or neuroprotective processes but have not been investigated in EAE. In subsequent studies, substances with clear neuroprotective properties will be combined with steroids, established immunomodulators and compounds developed in other WPs (e.g., compounds targeting Ncf, TREM-2 or TNFR signaling). Therapeutic effects will be investigated in vivo by clinical assessment, recording evoked potentials, and performing MRI measurements. Neurodegeneration and intracellular signal transduction will be evaluated by histopathological, histochemical and biochemical analysis.

In summary, it is expected that the results of the studies carried out in the Subproject Validation, focussing on animal models of MS, will have wide-reaching implications for other acute and chronic inflammatory CNS diseases and will stimulate investigations in other preclinical models (e.g., ischemia, traumatic injury, CNS infectious diseases) that are available in the consortium. Also, we expect that reagents developed in this Subproject will include promising new drug leads that merit further development towards clinical trials in patients with MS and possibly also other neuroimmune and neurodegenerative disorders.

Subproject Horizontal integration: ‘Coordination of Neuropathology and Genomics/Proteomics Reference Centers’
Coordinator: P5
Participants: P2b, P3, P9, P10, P14, P17

This subproject is aimed to provide the appropriate scientific and technological support to the consortium participants for those activities which involve neuropathological examination and high throughput genomics and proteomics analysis of relevant human and animal cell populations/tissue samples. This will be achieved by defining Neuropathology, Genomics and Proteomics facilities.
within the participants’ institutions. This subproject is also aimed to train personnel from the participants’ laboratories for optimal exploitation of the above methodological approaches.

1) The NeuroproMiSe Neuropathology Reference Center (NNRC) will be jointly organized by P2b and P5 in their respective laboratories at UKG-GOE and MUW, and will provide neuropathological support for the activities carried out within the subprojects “Identification”, “Mechanisms” and “Validation”. The NNRC activities will include:

   a) Provision of extensively characterized human autopsy and biopsy tissue for expression studies of newly identified target genes for MS susceptibility and phenotype. Partners P2b and P5 have collected a large repository of well characterized autopsy and biopsy tissue from more than 200 MS patients. A large collection of autopsy material of normal brain and other inflammatory brain diseases is also available. Immunohistochemical and in situ hybridisation analyses will be performed in relation to specific aspects of the lesions, like inflammation, demyelination, axonal/neuronal injury and remyelination.

   b) Creation of a genomic DNA bank from archival biopsy and autopsy tissue. Newly identified disease-related gene polymorphisms will be analysed in this material by PCR-technology and directly related to specific pathological alterations.

c) Reference center for experimental animal models. Since the clinical phenotype in EAE is determined by many different factors such as inflammation, demyelination and axonal injury, it is essential that a detailed neuropathological analysis, by quantitative technologies, is included in genetic studies on EAE susceptibility (Subproject “Identification”) and in studies related to molecular mechanisms of neurodegeneration (Subproject “Mechanisms”) and evaluation of therapeutic compounds (Subproject “Validation”). This will be achieved both by performing pathological analysis of nervous system tissue in direct cooperation with the involved partners and by training and education of the other partners in neuropathological analysis (conventional histology, immunocytochemistry, in situ hybridisation, confocal laser microscopy and electron microscopy) in the course of short term laboratory visits.

2) The NeuroproMiSe Genomic and Proteomic Reference Centers

Several of the partner institutes have well developed core facilities for modern methodologies in functional genomics and will provide scientific and technological support and training to the other consortium members. The genomics facility at ULUND (P3) and HPI (P14), and the proteomics facility at USOU (P10) will provide support for internal research groups and for their collaborators in the consortium to perform microarray and proteomic analysis in the model systems utilized within this project. The Biomedicum Biochip Center (BBC www.bioinfo.helsinki.fi), which is a joint operation between the Helsinki University and NPHI (P9), also provides microarray core facility services for both custom made and Affymetrix arrays. Both centers will provide biocomputing expertise as well as computing resources for the consortium partners to perform bioinformatics analysis.

This subproject also aims to integrate data from gene and protein expression studies performed in animal models and human systems. This will be achieved through integration in a genotype-phenotype data base developed by P17, which will be open to the consortium participants.

The further development of genomics tools will also take place within this subproject. With the objective of developing a new approach for linking molecular profiles to biology, specifically for defining pathways between biology and disease, the plasticity of algorithms and computer-based analysis tools will be exploited to organise genome-wide information collected from heterogeneous immune-mediated and neurodegenerative diseases affecting the CNS into an artificial neural network, and thereby create an in silico simulation of the CNS inflammatory response and disease-related pathways. It is expected that a new-generation information technology product will be produced that will have potential for the classification, prediction and treatment of neuroinflammatory diseases.

Since CNS tissue is by nature inaccessible and invasive diagnostic/prognostic procedures are not applicable, an artificial model where cellular function will be mimicked by algorithms should be of great value.

This neural network will be developed based upon the hypothesis that:
- CNS inflammation is a common and significant component in a wide range of otherwise heterogeneous CNS diseases having distinct pathological triggers, pathogenic mechanisms and clinical symptoms.
- Disease-specific components of each disease are to be found superimposed upon this.
- Aetio-pathogenic pathways will involve both of the above compartments.
- Potent protection and repair mechanisms will be common to all and concentrated in the area of overlap.

P14 has already collected the necessary gene expression data for initial algorithm development from four different immune-mediated and neurodegenerative pathologies affecting the CNS, using a 27K cDNA microarray which represents a significant fraction of the genes encoded within the mouse genome. Specifically, four well-established mouse models representing the three major diseases that affect the human CNS in developed countries were chosen, namely Alzheimer’s disease, multiple sclerosis and cerebral stroke. RNA/cDNA collection and experimental design was strictly standardized to meet MIAME criteria (minimal information about a microarray experiment, Brazma et al., Nature Genetics (2001) 29(4): 365-371) and to ensure that independent data sets can be compared and integrated. For example, the C57BL/6 genetic background was chosen because this is compatible with most experimental CNS disease models, and is the background of choice for the majority of transgenic and knockout animals, source tissue composition was common and flip-dye hybridizations of disease versus littermate, and disease versus universal RNA/cDNA controls were employed. In this project, P14 aims to utilize the high-throughput, time-course expression data generated from the expression profiling of each disease separately to construct a powerful algorithm (neural network) that will be built to robustly classify gene-expression changes into the ones that commonly characterize neuroinflammatory conditions and others that lead to and characterize specific brain pathologies. The network will then be further expanded, trained and tested using data sets generated from new mouse disease models developed and utilized by the consortium participants with the aim of increasing sensitivity and of obtaining a working platform that can differentiate between related and unrelated mouse diseases. The algorithm will be applied within the second part of the project for evaluating the efficacy of experimental therapeutic regimens.

6.2 DEMONSTRATION ACTIVITIES

Not applicable

6.3 TRAINING ACTIVITIES

The NeuroproMiSe Project will establish training activities aimed to:
- contribute to knowledge and technology transfer among all the participants of the NeuroproMiSe project;
- spread knowledge and technological processes outside the project;
- strengthen and integrate the research in Europe;
- promote new ideas in both basic and applied research;
- support a new generation of scientists able to translate research activities to the clinical practice.

The training programme of NeuroproMiSe will be managed in the Subproject “Training”. The training management is coordinated by participant P14, and involves P2a and the Coordinator or Co-coordinator of each Scientific Subproject (P3, P6, P19 and P5, respectively). The training management will report to the Steering Committee, where the final decisions on training and knowledge transfer are taken.

Two kinds of activities will be established: educational training and training workshops, covering scientific and technological matters as well as business and ethical aspects.

WP T1: Educational Training Program for internal staff training as well as for external training of young scientists. Internal staff training is performed as targeted training complementing the know-how of each partner with those of the other partners. Young scientists will be trained in the technological processes and knowledge developed. Supplementing Marie Curie Fellowships will be considered for
this matter. All cross-training in technological matters will be done between the partners periodically during the project, focusing in the technological aspects with relevance to their individual subprojects. Within the Consortium, the Lund training school will provide unique training opportunities in the use of mouse and rat models for MS. The Lund University (P3) already has extensive experience from training partly from having a Marie Curie training school for several years as described on their homepage http://net.inflam.lu.se/.

The Lund training school will provide up to date technology to:

i) identify genetic polymorphisms in complex genetics of animal model diseases.

ii) identify new pathways from findings obtained in the genetic studies through microarray technology, protein 2D analysis, bioinformatics, and statistical methods to calculate the effects and follow up results using biological experiments.

iii) use specifically designed mouse strains to investigate immune and inflammatory pathways.

Within the the NeuroproMiSe project, special attention will be devoted to exploit the expertise of several participants in gene expression technologies. Several laboratories in the consortium are actively involved in the application and further development of gene expression and targeting technologies in mice. For example, expertise in conventional methods of gene overexpression, knockout and knockin, as well as developing methodologies in conditional gene targeting (temporally and spatially regulated gene knockout and overexpression) and gene knockdown (transgene-based RNAi) are well established or currently being developed in the consortium (e.g. teams of P3, P4, P6, P7, P13, P14, P19). The consortium laboratories will coordinate to provide training and share knowledge within the project so that newly identified gene candidates emerging from the core subprojects can be functionally evaluated in mice as required.

WP T2: Training Workshops will be a second instrument used for training activities. The workshops will serve not only to communicate significant scientific results developed by each participant group but also to educate other researchers, investors, end users and potential licensors, to adapt the knowledge generated in the IP, and to learn about new methods and technologies as well as new potential products and their use. Through dedicated workshops, the scientific personnel of the project will receive additional training in matters related to exploitation of results, protection of intellectual property, ethical and gender issues. These workshops will be done periodically on a yearly basis, the first one to be done at the end of the first 18-month period.

Integration and cooperation with the teaching activities of the European School of Neuroimmunology (ESNI) will be ensured by the participation in the NeuroproMiSe Project of 4 members of the Scientific Board of ESNI (P5, P6, P8 and P14). P5, P6 and P8 also serve in the scientist panel of Neuroimmunology of the European Federation of Neurological Societies (EFNS), which is strongly committed to disseminate research results to the European Physicians Neurologists. NeuroproMiSe training activities will be also coordinated through the Department of Neurology of UKG-GOE (chaired by P2a), which is associated with an International Graduate School for MD-PhD in Neurosciences and participates in an EU-funded research training program for predoctoral neuroscientists. Furthermore, through the NeuroproMiSe website, all participants will be constantly informed on all relevant Scientific Meetings and Events, Conferences, Educational Courses, and European Summer Schools.
6.4 MANAGEMENT OF THE CONSORTIUM ACTIVITIES

NeuroproMiSe is built upon a consortium of 20 EU experts in the fields of neuroimmune diseases, genetics, immunology and neuroscience. The objectives of the project will be reached through 6 subprojects (four scientific and two administrative). Management activities are handled within the subproject Management.

The management of NeuroproMiSe is graphically represented below:

NeuroproMiSe will be managed through a General Assembly and a Steering Committee (SC). The General Assembly is chaired by the Project Coordinator Dr. Francesca Aloisi. Each contractor involved in the NeuroproMiSe project is represented by one person. Dr. Francesca Aloisi (ISS) will be the President of the SC and Dr. Harald Neumann (UNI-BONN), the co-coordinator of NeuroproMiSe, will serve as Vice-President of the SC. The presence of both a coordinator and co-coordinator has been decided by the Consortium to ensure not only the proper implementation of NeuroproMiSe activities and objectives but also to further stimulate and enforce the integration among the EU Countries Participants for all types of activities. The SC is composed by the coordinator and co-coordinator of each subproject, and by one business management representative (Dr. Giovanni Cozzone, ISS).

The General Assembly and the SC of the NeuroproMiSe Project will be supported by the Advisory Board. The Advisory Board will oversee the scientific, technological, innovative, regulatory, and
training activities of the project, with particular attention to ethical and society-related issues, including community involvement and gender issues. The Advisory Board will meet once a year or as required by the SC. The members of the Advisory Board are: Alastair Compston (UK); Roland Martin (Spain); Vijay Kuchroo (USA); Howard L. Weiner (USA); Hartmut Wekerle (Germany). All the AB members possess an outstanding knowledge and long-term experience in all the scientific activities of NeuroproMiSe and can, therefore, guarantee support to the SC to achieve the NeuroproMiSe objectives.

The General Assembly will decide the strategy of the project, including the programme of activities and budget allocation, will take key decisions in the work performed within the subprojects and will be the ultimate decision-making body if any conflict or dispute between the participants arise. The General Assembly is mandated with the task of ensuring that the project maintains its objectives and relevance within the research topic LSH-2004-2.1.3-2. The meetings of the General Assembly will take place once a year.

In compliance with the decisions of the General Assembly, the SC will be in charge of the scientific tasks and the technical assessment of the project. The President will convene the SC as often as the interests of the Consortium will require, and at least once every six months. The President will also convene meetings at any time, upon written request of any Contractor in case of an emergency situation, or upon request of 1/3 of the Contractors. The EU commission representative will have a permanent invitation to all SC and General Assembly meetings without voting power. The management activities are broken down into two main activities.

- Scientific management
- Administrative management

**Scientific management**

The SC, through the coordinator and co-coordinator, will implement the strategic orientation decided by the General Assembly, and will oversee both the scientific the administrative structure of NeuroproMiSe which are the executive arms of the project. The Subproject coordinators will coordinate the activity of the corresponding subproject to ensure that the scientific, technological, and training objectives will proceed as scheduled. Being active members of the SC, the Subproject Coordinators and Co-coordinators will report directly to the SC. The SC will constantly review the progress of the integrated project. This will be achieved by requesting each partner to provide every three month a report stating progress of the work and any deviations vs. plans. The Coordinator will forward to the Commission annual scientific reports, in addition to the annual cost statements. The SC will also inform the partners of relevant scientific work outside this IP, including complementary projects and other topics of LifeSciHealth in the FP6. The SC will oversee results delivery, to achieve in a timely and cost-effective manner the scientific and technological objectives of each subproject and related workpackages, as well as activities aimed at promoting integration among complementary tasks of the different subprojects. By monitoring the project activities, especially the plan of use, exploitation and dissemination of the results, the SC will ensure not only maximal integration and synergy between subprojects, but also an adequate and updated validation of milestones and deliverables.

The scientific units of the NeuroproMiSe project will meet every six months to discuss the results achieved and the plans for the forthcoming year. The kick-off meeting (month 1) will be dedicated to discuss the first 18 months of the project.

**Administrative Management (Business and Project Management)**

The team headed by the Coordinator, in collaboration with a Professional Accounting Firm, retained as a consultant for the Istituto Superiore di Sanità (ISS), and the Administrative Services of the ISS, is responsible for the overall financial and administrative management of the NeuroproMiSe Consortium. Through the Coordinator, the administrative management will ensure communication between the Commission and the Consortium. The ISS team is assigned with the duty to complete all types of reporting concerning the financial and administrative aspects to be addressed to the EU Commission, forward the allocated budget to the partners of the Consortium, transfer funds and prepare financial data for detailed implementation plans. In addition to drafting and preparing
intermediate and final progress reports, cost statement support to technical and financial audits, cost statements (summary expenditure report: Audit certificate/external independent auditor, Management justification, Summary Certified Statement), it will introduce and train the partners of the consortium, especially the project leaders, on accounting principles and contractual rules of the consortium contract.

Business management

Dr. Giovanni Cozzone, an expert in the Business field and a key consultant for the Italian National Institute of Health (Istituto Superiore di Sanità, ISS) is responsible for the Business management. He will oversee the overall legal, contractual, intellectual property actions and technology transfer activities of NeuroproMiSe, including the EU Commission contractual commitments. He will be in charge of preparing and updating the Consortium Agreement among the parties of NeuroproMiSe, ensuring proper accounting and financial management, and preparing accounting and financial statements for each reporting period. He will report to the Steering Committee. In addition, he will coordinate decisions by the SC and/or Contractor(s) on filing of patents and patent-searches or other IPR applications.

A Professional Accounting Firm, to be hired immediately after the contract signature, will assist the coordinator in management activities including: implementation of proper accounting and reporting procedures; development of an archiving, data retrieval, economic, accounting, and financial reporting system; interim economic and financial statements on the use of funds; pro-forma and final statements for the reporting period; 18-month rolling forecasts for each reporting period; reconciliation statements versus budget; preparation of audit certificates; selection of a certified public accountant to certify the cost statements for each reporting period; assembly of the economic and financial reports of the Consortium to the EU Commission for each reporting period (see also Appendix A, section A.2).

For the preparation of annual audit certificates, some of the Contractors with the AC, FC and FCF cost models will resort to subcontractors, i.e. external auditor or a competent public officer from an organization other than the contractor.

Project management

Ms. Estella Sansonetti will assist Dr. F. Aloisi in all the management activities. She will be the contact person for all administrative activities between the coordinator, the co-coordinator and the NeuroproMiSe participants. She will file all official documents and the reports relative to the activities of the NeuroproMiSe meetings, being responsible for real-time communication, dissemination of information and interaction among the Coordinator, the Co-Coordinator, the Steering Committee, and the partners. Ms. Sansonetti will also organize meetings and events and other communication strategies (e.g. through the NeuroproMiSe website). One additional, project management staff member will be supported by EU funds.

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Administrative Services. The Office of Administrative Affairs and Financial Resources (Responsible Director: Dr. Rosa M. Martoccia) is the legal administrative service of the ISS and will be involved in the transfer of funds received from EU. Depending on the funds assigned, this Office will transfer the specific amount to the Consortium participants.

Data Management Services. The Data management, documentation, library and editorial services will be the technical consultant for the NeuroproMiSe Project for the development of information systems on health and research activities.

Communication and dissemination of information will be supported by a dedicated, primary domain web site, which will be also used in the overall management, in accordance with the Coordinator and Steering Committee of NeuroproMiSe.

The Management of the project (Scientific and Administrative) is described in more detail below.
6.B PLANS

6.5 PLAN FOR USING AND DISSEMINATING KNOWLEDGE

After the launch of the NeuroproMiSe project, an Intellectual Property Protection and Exploitation Policy (IPPEP) will be developed and submitted to the Steering Committee for approval and immediate adoption by the Consortium.

The IPPEP is based on the following cornerstones:
- patent filing criteria and invention reporting procedures;
- adequate and effective protection of patentable knowledge by patent filing in all major patent countries and regions for sole and joint inventions and related measures in the event that the originator(s) do not intend to seek protection for the specific invention;
- criteria for rights of first refusal in favour of industrial participants to invention with no distinct ownership or inventions developed by academic participants;
- assistance to participants seeking help in protection of sole and joint inventions;
- technology marketing to select and acquire strategic industrial partners for the further technical and commercial development of specific inventions;
- license and cross-license agreement assistance to partners seeking help in establishing exploitation agreements;
- protection of rights of academic participants in specific inventions.

A dissemination plan for new knowledge generated by NeuroproMiSe activities beyond the consortium and during as well as after the project lifetime, will be decided by the Steering Committee soon after the start of the project. This will take into account the actions taken and parameters used for IP protection of new knowledge. Thus, clearance for dissemination of new knowledge will be given by the Steering Committee and will be regulated in the Consortium Agreement. Once clearance is obtained, new knowledge will be disseminated through the NeuroproMiSe web-site, scientific meetings and publications in scientific journals, reports to European and international organizations, participating institutes and industries.

6.6 GENDER ACTION PLAN

The NeuroproMiSe proposal has been prepared with special attention to ensure an adequate representation of women in all aspects of the project. About 50% of the personnel involved in the NeuroproMiSe project are women. Women in leading positions in the project represent 15.0% of the participants, and two out of eight members of the Steering Committee (25%) are women.

Immediately after the start of the project, a ‘gender awareness’ programme will be designed to educate all consortium members to the policy of Gender Equality in European research. The first action will be to summarize and deliver to all participants, through the NeuroproMiSe website, the relevant information concerning the Commission’s Progress Report of May 2001 on the work of the Helsinki group, and the European and National Reports on the situation of women scientists which are published in the EU website, along with references to other pertinent websites and documents. This will be followed by ‘gender sensitisation’ programmes performed by gender and society experts contracted to give seminars on the role of gender in our society. Within the series of yearly thematic workshops to be organized by the consortium, one will include gender and society experts. Following this, ‘gender monitors’ will be asked to volunteer locally to each partner, to ensure continuous development and increased awareness of gender issues in the workplace. A gender expert will be asked to monitor annually the gender dimension of the project.

In addition, the awareness and care of the partners concerning gender equality will be implemented with the following actions:
1) increase the participation of female researchers in the consortium activities and ensure a balanced participation of women in training (50%);
2) support career advancement of female junior researchers within the project;
3) consideration of the potential impact of all decisions within the project on gender equality;
4) use of gender-neutral language in meetings, official documents and correspondence;
5) creation of a Task Force within the Consortium on Gender Equality. Each subproject coordinator and co-coordinator will be responsible for proposing annual gender equality activities with respect to the tasks of the subproject and related workpackages (planning and results). Based on this, the Task Force Gender Equality finalises the annual update of the overall gender action plan. The review of success and weak points will be done by the Task Force Gender Equality and will be part of the regularly updated gender action plan. The results and updates of the gender action plan shall be part of the agenda of the General Assembly and Steering Committee meetings, in order to promote the awareness of the importance of gender issues in this project.
6) targeted address of job vacancies to female researchers and encouraging their application. In order to achieve a broad dissemination within the female scientific community, job advertisements will be published through the National Networks of Women Scientists and with the help of the virtual platform 'European Platform of Women Scientists'.
7) check the possibility of part-time work in order to increase participation of persons with family responsibilities and to provide incentives for persons to return to work following maternity/paternity leave, including the investigation of opportunities for flexible work practice (e.g. teleworking).

These actions will be coordinated and managed by the NeuroproMiSe Steering Committee to ensure that all the above activities are adhered to.

MS is more prevalent in women than in men (with a ratio higher than 2:1); hence, the development of novel therapeutic compounds for this disease would represent an important progress in addressing women’s needs for MS.

6.7 RAISING PUBLIC PARTICIPATION AND AWARENESS

To contribute to spreading of knowledge and public awareness of the scientific activities and results related to the NeuroproMiSe project, the following activities are foreseen:

- The NeuroproMiSe web-site will include a ‘public section’ which will be open to the non scientific community and will be updated with lay-language information concerning the purposes and benefits of the studies performed in the NeuroproMiSe project. Presentation of the NeuroproMiSe project to the public will include general information on the consortium composition, scientific aims of the project and planned activities. Reports on annual workshops and General Assembly meetings held within the NeuroproMiSe project will be also published in the NeuroproMiSe website.
- Publications in international journals will allow dissemination of the results obtained within the NeuroproMiSe and will acknowledge funding form the European Commission.
- Government health sites in the nations participating to the project will be used as a tool for spreading awareness of the results obtained in the NeuroproMiSe project (for example, the Istituto Superiore di Sanità in Italy (www.iss.it), Karolinska Institutet (www.ki.se) in Sweden, etc.)
6.C MILESTONES

6.C MAJOR MILESTONES OVER WHOLE PROJECT DURATION

The ultimate goal of NeuroproMise is to develop neuroprotective/immunomodulatory compounds and therapeutic strategies to counteract the neurodegenerative process underlying disease progression and disability in MS and other neuroinflammatory diseases. In order to achieve this goal, the following major milestones (MM) and deliverables (DE) have been identified within each subproject (please note that the numbering used here is not related to the numbering used in the individual workpackages).

MM-I1 (Subproject Identification) Identification, through genetic studies in congenic rodent strains and genomic and proteomic approaches in animal models of neurodegeneration, of genes and molecular pathways that determine susceptibility to immune-mediated CNS disease and underlie neuronal vulnerability (month 24/36). The role of candidate genes and proteins will be then validated in appropriate in vivo/in vitro experimental models as well as in human brain samples from MS patients (month 36/48). The results obtained in experimental animal models will provide the basis for the design of genetic studies to be performed in a well characterized and homogeneous cohort of MS patients and for the development/identification of compounds able to interfere with the activity of the candidate genes/pathways.

DE-I1.1: Selection of minimized congenic mouse and rat strains containing less than 10 genes that control essential pathways of EAE and neurodegeneration, and of finalized partial advanced intercross lines with defined interchromosomal and extrachromosomal interactions between defined candidate genes (mo 24)
DE-I1.2: New positionally cloned genes harbouring polymorphic effects on EAE development and neurodegeneration (mo 36)
DE-I1.3: Global gene expression profiles and fully analyzed data sets listing key genes and pathways contributing to initial and late stages of axonal degeneration (mo 24)
DE-I1.4: Proteomic profiles of normal axon-derived material and axons undergoing degeneration at selected (early and late) time points (mo 24)
DE-I1.5: Definition of biomarkers for axonal degeneration in different experimental settings (mo 36)
DE-I1.6: Identification of new targets for neuroprotective therapies (mo 36)
DE-I1.7: New models for MS and neurodegeneration based on genetic, genomic and/or proteomic discoveries (mo 48)
DE-I1.8: New drugs affecting the activity of genes and gene products involved in EAE susceptibility or neuronal vulnerability (mo 24 to 60)

MM-I2 (Subproject Identification) Identification of genes underlying disease susceptibility in a homogeneous and well characterized cohort of Finnish and Swedish MS patients. This will be achieved through subsequent steps involving completion of physical data-set and data-base structure of more than 4,000 individuals (patients and controls) containing phenotypic information, genotyping for known risk genes and gene-gene interaction analysis in MS patients (mo 24). These steps will set the basis for testing of new candidate risk genes for MS identified through comparative genomic approaches (MM-I1). High-throughput SNP genotyping with densely spaced markers (1 per 1-2 kilo base pairs) will then be performed for selected, candidate genes.

DE-I2.1: Establishment of a database structure and dataset of more than 4,000 individuals (2,000 patients, 2,500 family members and controls) containing phenotypic information (mo 9)
DE-I2.2: Full genotypic characterization and report of gene-gene interaction analysis of known MS risk genes in the above data-set (mo 24)
DE-I2.3: Report on newly identified genes involved in susceptibility to MS, and on gene-gene and gene-environment interaction analysis in MS patients (mo 48/60)
DE-I2.3: Report on correlation of the genetic information with identified patterns of tissue damage in MS patients (mo 36/48)

MM-M1 (Subproject Mechanisms): Identification of immunopathological mechanisms involved in MS and neurodegeneration with the purpose of designing rational ways to interfere with them. This goal will be reached through the development of improved experimental models (mo 24) that will allow to test several hypothesis concerning the most critical pathways involved in immune-mediated (T cell-, B cell-, and macrophage/microglia-mediated) CNS tissue damage and will be useful for preclinical testing of therapeutic compounds within this and other subprojects.

DE-M1.1: Humanized transgenic mouse models for the study of CD4+ and CD8+ T cell influences on EAE development and neurodegeneration, and for evaluation of therapeutic compounds (mo 18/24)  
DE-M1.2 Compounds (e.g., soluble MS-associated TCR receptors and MHC molecules, DR15-MBP tetramers, and others to be identified or developed) suitable for modulating EAE development and associated CNS tissue damage (mo 12/24)  
DE-M1.3: Lentiviral vectors for the in vivo and in vitro transduction of cytokines/chemokines implicated in chronic CNS inflammation and generation of new animal models mimicking abnormal lymphoid neogenesis observed in MS patients (mo 12/24)  
DE-M1.4: New knowledge on the pathogenetic role of ectopic lymphoid follicles in MS (mo 24)  
DE-M1.5: Demonstration of the disease modifying effects of compounds regulating MHC epistatic interactions and lymphoid neogenesis (mo 24/36)  
DE-M1.6: Markers of axonal transport disturbance/failure following destructive neuron/microglia interactions (mo 36)  
DE-M1.7: Identification and validation of molecules and pathways involved in CD4+ T cell-, CD8+ T cell-, antibody- and microglia-mediated tissue damage (mo 48)  
DE-M1.9: Report on the therapeutic efficacy of compounds acting on newly identified molecular targets involved in innate and adaptive immune responses targeting the CNS (mo 48/60)

MM-V1 (Subproject Validation) Development and/or testing of new pharmacologic tools for the regulation of molecular pathways (TREM-2, Ncf-1, TNF/TNFR subtypes, apoptosis, ionic channels, glutamate receptors) involved in neuroinflammatory processes and neurodegeneration. Initially, a number of candidate compounds, already identified for their anti-inflammatory and/or neuroprotective activity, will be investigated with the purpose to improve our knowledge of their mechanism of action and establish protocols for their use in preventive and therapeutic settings, as well as in combination therapies (mo 24/36). Validation of new therapeutic tools will also involve the development of improved animal models (humanized TNFR subtype transgenic mice and EAE models with optic nerve degeneration) (mo 12/24). At a later stage, this subproject will focus on new targets for therapy identified through the genetic, genomic and proteomic studies performed in the Subproject Identification.

DE-V1.1 New patented compounds acting on pathways regulating inflammation (Ncf-1 pathway modulators, TNFR subtype agonists and antagonists) (mo 24)  
DE-V1.2 New CNS- and neural cell-penetrating compounds with anti-apoptotic and neurotrophic activity (TNF muteins, TAT fused to anti-apoptotic molecules and neurotrophic factors) (mo 24)  
DE-V1.3 New compounds affecting ionic channel and neurotransmitter function in neurons and displaying improved neuroprotective activity (mo 24/36)  
DE-V1.4 New targets useful for therapy identified through the analysis of Ncf-1 and TREM-2 pathways (mo 24/36)  
DE-V1.5 New EAE models with strong neurodegenerative aspects for neuroprotective treatment evaluation (mo 12)  
DE-V1.6: New humanized transgenic mice and in vitro systems to evaluate the neuroprotective role of compounds modulating TNF/TNFR pathways (mo 24)  
DE-V1.7: Report on the therapeutic (neuroprotective) efficacy of existing and newly identified compounds in different experimental settings (in vitro, semi-in vivo and animal models) (mo 24 to 60)
DE-V1.8: Genetic biomarkers to monitor the anti-inflammatory/neuroprotective effects of new drugs (mo 24 to 60 mo)
DE-V1.9: Report on the therapeutic efficacy of combined anti-inflammatory/neuroprotective strategies (mo 24 to 60)

MM-HI.1 (Subproject Horizontal Integration). Organization of Reference Centers, open to all consortium participants, providing adequate support, coordination and appropriate technological platforms for neuropathological, high throughput genomic and proteomic studies. The two Neuropathology Reference Centers at UK-GOE (P2b) and MUW (P5) will be established for collection and analysis of brain tissue samples from MS patients and animal models, and for providing technical support and expertise in neuropathological techniques. The Genomic Reference Centers at U-LUND (P3) and NPHI (P9) and the Genomic Reference Center at USOU (P10) will provide support for the identification of new disease-associated genes and proteins, and for the standardization of technical procedures and data analysis. The technical platform established at HPI (P14) will allow the organization of genome-wide information collected from heterogeneous neuroimmune-mediated diseases into an artificial neural network to create an in silico simulation of the CNS inflammatory response and disease-related pathways useful to monitor the effects of therapeutic compounds.

DE-H1.1 DNA isolated from archival biopsy and autopsy MS tissue to be used for high throughput SNP analysis of candidate risk genes and association with different lesion patterns (mo 18)
DE-H1.2 Quantitative characterization of MS brain lesions in biopsy and autopsy material in relation to neuropathological parameters and disease phenotype (mo 36)
DE-H1.3 Validation of the expression pattern of disease relevant genes/proteins identified in the other subprojects and correlation with specific pathological phenotypes of MS disease (mo 24 to 60)
DE-H1.4 Gene and protein expression profiles of animal models of disease: basic patterns and modified patterns associated with therapeutic treatments (mo 24 to 60)
DE-H1.5 Fully validated computer-based algorithm representing gene changes associated with different mouse models of inflammatory neurodegeneration (mo 24)
DE-H1.6 Application of algorithm to the evaluation of data sets derived from studies assessing the outcome of experimental therapeutic regimens (mo 24 to 60)

MM-T1 (Subproject Training) Organization of a Training task force whose activities are aimed at: i) defining an educational training programme to promote the synergies of the cooperating scientific groups; ii) attracting young scientists and supporting them through the Marie Curie Fellowships; iii) training the scientific personnel in aspects related to IPR, result exploitation, ethical and society issues; iv) disseminating scientific results developed within the IP.

DE-T1.1 Presentation and continuous updating of the training programme in agreement with the principal tasks of the project (including exchange scientific courses, calls for candidates for Marie Curie Fellowships, specific courses, thematic workshops, technical meetings, short term personnel exchange for training in Neuropathology, genomics and proteomics technologies) in the EuroproMiSe website (mo 10 to 60).
DE-T1.2 Continuous updating on EU and International Courses, Workshops and Meetings related to issues relevant for the project, through the EuroproMiSe website (mo 7-60)
DE-T1.3 Coordination with the activities of other International and EU training initiatives and programmes (International Graduate School for MD-PhD in Neurosciences associated with Göttingen University, ESNI, EFNS) (mo 7-60)
DE-T1.4 Organization of specific technical meetings and thematic workshops on selected issues relevant to the project (mo 12 to 60)
DE-T1.5 Calls for candidates for Marie Curie Fellowships (18 mo)
DE-T1.6 Yearly courses on functional genomics, proteomics and neuropathology (mo 12 to 60)
DE-T1.7 Short term personnel exchange among partners (mo 12 to 60)
DE-T1.8 Thematic training on exploitation of results, IPR and patents issues, ethical issues (particularly animal experimentation) and gender issues (mo 12 to 60)
DE-T1.9 Yearly workshops to communicate the scientific results developed by each participant group and to train other researchers and potential stakeholders on the knowledge, methods, technologies, as well as new potential products generated in the IP and their use (mo 12 to 60)

MM-MAN1 (Subproject Management) Coordination of all the project activities within the consortium and towards the EC, including Science, Business and Administrative Management.

DE-MAN1 Neuropromis meetings: Steering Committee (every 6 months) and plenary (annual) meetings (months 1-60)
DE-MAN2 Project presentation (mo 3)
DE-MAN3 NeuroproMiSe website for coordination of the activities within the consortium, knowledge dissemination, and presentation of the training programme (mo 6-60)
DE-MAN4 Annual Report to the EC and certified costs (every 12 mo)
DE-MAN5 Detailed implementation plan (every 18 mo)
DE-MAN6 Plan for using and disseminating knowledge (mo 6-60)
DE-MAN7 Gender action plan (mo 6-60)
DE-MAN8 Monitoring of science- and society-related issues, including community involvement and ethical issues related to project activities (mo 1-60)
DE-MAN9 Report on raising public participation and awareness (mo 18-60)
7. PROJECT MANAGEMENT

7.1 MANAGEMENT STRUCTURE

The NeuroproMiSe Consortium is composed of 18 contractors, with a total of 20 actively participating research groups from 9 EU Member States. The coordinator of the consortium is the Istituto Superiore di Sanità/ISS (Dr. F. Aloisi), Italy, together with the co-coordinator University of Bonn/UNI-BONN (Dr. Harald Neumann), Germany.

The project management structure and functions are graphically represented below:

Decision-making, advisory and executive bodies

The General Assembly (GA) is the governing body of the Project and will take decisions in each NeuroproMiSe-related issue. The GA is chaired by the project coordinator Dr. F. Aloisi and is composed of one representative for each contractor. Each representative has one vote and may appoint a substitute to attend and vote at any meeting of the GA. The meetings of the GA take place once a year. The General Assembly shall in particular be entitled to:

1) decide upon major changes to the Program of Activities and to the Budget, if exceeding 30% of the EC contribution;
2) decide upon staggered payments of the contribution to a Contractor for justified reasons;
3) propose to Contractors review and/or amendment of terms of the EC Contract and the Consortium Agreement;
4) decide to suspend all or part of the Project or to terminate all or part of the Contract, or to request the Commission to terminate the participation of one or more Contractors;
5) in case of default of a Contractor, agree on actions to be taken against the Defaulting Contractor;
6) decide upon the enlargement of the Consortium and the access of new Contractors to the Contract and the Consortium Agreement;
7) settle any dispute arising from the Project implementation.

Consultations and reports from an independent panel of scientists, the Advisory Board (see below), will be available to the GA (as well as to the Steering Committee) for decision-making purposes.
The voting mechanisms: The GA may validly meet if two thirds (2/3rds) of its members are present or represented. In case quorum is not met and in the need of urgent deliberations, the GA may be convened once again within no more than three (3) weeks from this date, and may validly deliberate even in the absence of the quorum. In case of written consultation, the letter of the Chairman is required to specify the deadline for the required response. Failure to respond within this deadline will be deemed as a non-vote for the application of the quorum and majority vote requirements. Each member of the Bodies will have one (1) vote. In case of a draw, the President will have a casting vote, Decisions will be taken by the majority of the votes of the members present at a quorate meeting on the majority of items being discussed, with the exception of agenda items related to: i) dissemination of new knowledge from the project not to be used by the members and ii) proposals to serve notices on a defaulting member. On the latter items, decisions will be taken by a qualified majority of two-thirds (2/3) of all Members. Inclusion, withdrawal or exclusion of a Contractor requires unanimous vote minus that of the member concerned. Any decision requiring a vote will be identified on the pre-meeting agenda, unless there is unanimous agreement to vote on a decision at that meeting and the 100% of the members are present or represented. Minutes of the meetings will be transmitted to members within 30 calendar days from the concerned meeting and will be considered accepted by the GA, if no member has objected in a traceable form to the President within 15 calendar days from receipt.

The Advisory Board
The General Assembly and the Steering Committee of the Neuropromise Project will be supported in their decisions by the members of the Advisory Board (AB). The AB is responsible to provide advice, upon request, on specific issues, including: scientific and technological development of the program to reach the objective in a timely fashion; dissemination and exploitation of the results within and outside EU; society related-issues including community involvement and gender issues; industrial exploitation of Neuropromise products; and scientific networking. The AB will meet once a year or as required by the GA. Scientists who have already agreed to join the Neuropromise AB are:
Alastair Compston, Professor of Neurology, Cambridge Center of Brain Repair, Cambridge, UK
Roland Martin, ICREA Research Professor, Hospital Universitari Vall d’Hebron, Barcelona, Spain
Vijay Kuchroo, Associate Professor of Neurology, Department of Neurology, Harvard Medical School, Boston, USA
Howard L. Weiner (USA), Professor of Neurology, Department of Neurology, Harvard Medical School, Boston, USA
Hartmut Wekerle, Head of Neuroimmunology Unit, Max-Plank Institute of Neurobiology, Germany
All the AB members possess an outstanding knowledge and long-term experience in all the scientific activities of Neuropromise and can, therefore, guarantee support to achieve the Neuropromise objectives.

The Steering Committee
The Steering Committee (SC) is the operational body of the Project. The SC will execute the decisions taken by the General Assembly through the Science and Business management teams (see below). The Coordinator of the Project, Dr. Francesca Aloisi (ISS) will be the President of the SC and Dr. Harald Neumann (UNI-BONN), the Project Co-coordinator, will serve as Vice-President of the SC. The SC is composed of 9 members in total, the Subproject Coordinators and Co-coordinators (8 members) and one expert responsible of the business management activities (Dr. Giovanni Cozzone, ISS).
Dr. Francesca Aloisi is group leader at the ISS, has been PI in several National Projects, and is currently the coordinator of a National Project on ‘Novel immunomodulatory and neuroprotective strategies in experimental models of MS’ (1ACF) and PI of a joint NIH-ISS Project on ‘Immunopathogenesis and therapy of MS’. She serves as a member or reviewer in several scientific advisory boards of National (President of AINI) and International Societies (member of ISNI), Research Institutions (reviewer for MRC) and Foundations (reviewer for ARSEP, Myelin Project; member and reviewer for FISM). As member and chair (7th International Congress of Neuroimmunology, Venice, Sept. 28- Oct. 2, 2004) of Organizing committees and conference scientific advisory boards, she has gained experience in the organization of national and international scientific conferences.
Dr. Harald Neumann is group leader at the University of Bonn. He has a degree in medicine and business administration. He has been PI in several National Projects and member of the Managing Board of the Institute of MS Research in Göttingen (funded by the Hertie-foundation and the University of Göttingen). He serves as a reviewer and advisor of International Societies (MS Society UK, MS Society Canada, Hertie-Foundation Germany and FISM, Italy). He gained experience in the organization of national conferences (German Neuroscience Conference) and international workshops (International Congress of Neuroimmunology, Montreal 1998 and Venice 2004).

The members of the SC will meet to review progress and comment on the future activities and proposed financial plan of NeuroproMiSe as put forward by the General Assembly. The primary function is to develop policies and long range strategic plans for NeuroproMiSe to be proposed to the General Assembly and to develop plans for execution and to instigate, instruct and approve financial/resource distribution. The President will convene the SC as often as the interests of the Consortium will require, and at least once every six months. The meetings of the SC will be held via physical meetings or teleconferencing. The SC will be responsible for:

- implementing the strategic orientation decided by the General Assembly;
- implementing and updating the Program of activities;
- monitoring the Project Deliverables;
- supporting the Coordinator in elaborating reports on the whole Project;
- proposing to the General Assembly any amendments and integrations of the Project and the Program of Activities;
- agreeing on press releases and joint publications of the Contractors with regard to the Project;
- making proposals on the policy of dissemination and exploitation of the results, including patent application and IPR related matters on the basis of the Legal and IPR Board and of the Ethics Steering Group advice;
- making proposal to the General Assembly for the review and/or amendment of the terms of the Contract or to request the Commission to terminate the participation of one or more Contractors;
- reviewing and deciding in case of default of a Contractor, the assignment of the Defaulting Contractor's tasks, to another Contractor or to any new entity to join the Project for that purpose;
- making proposals to the General Assembly to suspend all or part of the Project or to terminate all or part of the Contract, or to request the Commission to terminate the participation of one or more Contractors;
- approving the changes to the budget, if the amounts transferred do not exceed 30% of the EC contribution.

In addition, the SC will be responsible for the business management activities of the consortium through a business management representative, Dr. Giovan Battista Cozzone, who will be responsible of the Business Management Team (see below) and will report to the SC every six months.

The Business Management representative in the SC will be in charge of:

- supporting the Coordinator in order to fulfill his obligations, including preparation of the Coordinator cost statements, day-to-day business and administrative support functions.;
- implementing the decisions of other Bodies of the Project;
- carrying out the strategic management of the Project including negotiation with the Commission regarding budgetary aspects, negotiation of Consortium Agreement and review of resource status;
- carrying out the financial management of the Project, co-ordination and approval of the collective cost statements;
- making proposals and monitoring the correct budget allocation to each activity and the related budget sharing among partners, Subprojects and WPs;
- supervising the financial issues on all partners;
- making budget-related proposals to the General Assembly according to the rules for the management of the funds received from the Commission.
- dealing with the issues related to intellectual property rights and patenting policy and in particular providing assistance to the Project Bodies on all legal aspects concerning the Consortium Agreement implementation and in particular on the aspects relating to the IPR.
matters (for instance: protection, access, use, joint ownership).

Scientific management
The Scientific management team is composed of all Subproject coordinators and co-coordinators and will report directly to the SC. The Scientific management team will coordinate the activity of the corresponding subprojects to ensure that the scientific, technological, and training objectives will proceed as scheduled. The Scientific management team has the general task of ensuring that the project maintains its objectives and relevance within the research topic LSH-2004-2.1.3-2 and will constantly review the progress of the integrated project. This will be achieved by requesting each partner to provide every 3 month a report stating progress of the work in comparison to the contract. The Coordinator will forward to the Commission annual scientific reports, in addition to the annual cost statements. The Scientific management team will:

- Oversee knowledge flow within the Consortium through monitoring the progress of the project by preparing, validating and circulating results of the achieved milestones and of the achieved deliverables.
- Coordinate integration activities, aimed at promoting integration among complementary tasks of the different subprojects and workpackages, and researcher skills through mobility and training.
- Oversee training activities to ensure improvement of knowledge and skills, both within and outside the consortium, through an integrated training programme;
- Oversee dissemination activities aimed to inform the scientific community of the significant scientific results developed by each participant group within the different subprojects. This will be achieved by organization of annual workshops and by promoting publication of the participants’ work in peer-reviewed journals of basic, neurological or general medical sciences, as well as seminars and oral and poster presentations in national and international conferences. Special focus will be given to establish contacts with local Science/Biotech organizations, like Bioregions. The objective is to stimulate the dissemination of the IP results to the whole range of target groups/stakeholders. The Innovation Relay Centre Network tools of the EU will be used to disseminate results within Europe. Knowledge flow and dissemination activities will be supported by the NeuroproMiSe web-site (see below).
- Assist the SC and Contractors in the identification of all the results arising from the project activities those that could be subject of industrial and commercial exploitation.
- Monitor the development of Intellectual property, aimed at promoting the development of new bio-therapeutics. Protection of Intellectual property will be coordinated by the Business Management Team, the latter being responsible for providing all legal and expert advice on intellectual property protection and technology transfer activities. The consortium agreement will cover the general rules of IPR strategies and exploitation routes and will be, if necessary, amended during the project life time.

The NeuroproMiSe web-site
To ensure adequate planning, direction, scheduling, monitoring and dissemination of the NeuroproMiSe activities, the management of the Consortium will be supported by a web-based communication platform, the NeuroproMiSe web-site, which will be used as a management and dissemination tool. The website will include: i) presentation of the NeuroproMiSe project; available to the public, providing general information about the consortium composition, the scientific aims of the project and the planned activities; ii) the NeuroproMiSe management section: a dedicated intranet for the consortium participants, password protected with several layers of security and access; it will include several subsections for uploading and downloading documents to be shared among members, training and IPR information, official scientific meeting minutes, etc; iii) NeuroproMiSe Conferencing Forum: this is a password protected video-conferencing resource. Its aim is to provide a service to all NeuroproMiSe partners. It will be used to organize videoconferences for special needs, such as discussion of recent research results, training activities and planning.
A professional web-site developer firm will be hired to develop a dedicated primary domain website to be used in the overall management of the Project, in accordance with the Coordinator and SC.
Administrative Management (Project and Business Management)

The administrative and business management team will provide support and services to the SC and Subproject Leaders. This team is assigned with the duty to complete all types of reporting concerning the financial and administrative aspects to be addressed to the EU Commission, forward the allocated budget to the partners of the Consortium, transfer funds and prepare the financial data for detailed implementation plans. Specifically, it will be responsible for:

- Yearly financial and accounting reports for each reporting period, including cost statements to the SC and the EU Commission;
- Timely delivery of accounting reports by the Contractors;
- Organizing audits initiated by the Commission;
- Executing the decisions taken by the General Assembly for immediate resolution of any conflict between or among the participants;
- Developing strategies for NeuroproMiSe activities and funding;
- Executing administrative and financial decisions adopted by the SC;
- Ensuring a permanent contact point with the NeuroproMiSe participants for all the administrative procedures;
- Maintaining an archive of all Consortium documents.

NeuroproMiSe management administration

Besides the SC President, Francesca Aloisi, the Administrative Team will be composed by:

Estella Sansonetti, Istituto Superiore di Sanità. She will coordinate the NeuroproMiSe Administration Office and will be the contact person for all the administrative procedures between the coordinator/co-coordinator and the NeuroproMiSe participants. She will be responsible for the correspondence and for sending minutes from the GA and SC meetings to all other partners without any delay. Ms. Sansonetti will also organize meetings and events and other communication strategies. She will have a daily contact with the Coordinator Dr. Francesca Aloisi and will be reporting to her. Ms Sansonetti has been working with Dr. F. Aloisi since 10 years and has been in charge of the secretarial staff managing national scientific projects coordinated by Dr. Aloisi and other scientists in the Neurobiology and Neurophysiology Units at the ISS. She has organized international meetings and conferences and supports F. Aloisi in any collaboration with other national and international laboratories. One additional full-time staff administrative member will be supported by EU funds.

For the coordination of the accounting activities of the Consortium members and the assembly of accounting reports for the Consortium and the EU Commission, a Professional Accounting Firm, in the role of subcontractor, will provide the appropriate accounting technology infrastructure, and cost statement and finance management services (described in detail in Appendix A, section A.2). For the preparation of audit certificates, some of the Contractors will resort to subcontractors, i.e. external auditor or a competent public officer from an organization other than the contractor.

Dr. Rosa M. Martoccia: Director of the Office of Administrative Affairs and Financial Resources, the legal administrative service of the Istituto Superiore di Sanità (ISS). This Office will receive the entire financial contribution from the Commission and manage this contribution by transferring it to the Contractors in accord with the Programme of Activities and the decisions taken by the appropriate bodies.

Business management

Dr. Giovanni Cozzone, the business management representative in the SC, will be responsible for the business management team and additional back-office support to be hired as needed. Dr. Cozzone will work in close collaboration with the Administrative and Financial Resources Management of the Istituto Superiore di Sanità, and will report to the SC. Dr. Cozzone is a corporate finance, technology transfer and strategic partnering specialist with over 25 years of business experience encompassing large corporations, start-ups and venture capital firms. Professional experience includes 8 years as an executive in a global pharmaceutical company and 15 years in several Italian and International start-ups. Skilled at creating and growing fast-paced organizations, Dr. Cozzone has international experience on both sides of the Atlantic, network in the European business and Corporate Finance sectors, diversified science and technology background, solid oral and written communication skills,
strong and creative negotiation skills. Currently, he heads, with the role of expert advisor to the President, the technology transfer and industrial collaboration activities of ISS. He is currently in charge of the Business Management of AIDS Vaccine Integrated Project’’ Acronym (“AVIP”), an EU funded Integrated Project coordinated by the ISS.

The tasks of the business management will be:
- To structure, execute and policy the consortium agreement among the participants;
- To coordinate protection of any new knowledge and intellectual property identified by the SC and/or Contractors as potentially patentable;
- To structure, execute and policy the agreements with third parties;
- To coordinate the financial and economic management of EU funds; this task will be supported by outsourcing the services of a professional accounting and financial firm that will provide all the IT and accounting resources needed by the consortium.
- To monitor due diligence of members, financial practices and reports.
- To timely assemble economic/accounting reports for the SC and EU Commission.

Gender action plan
The Management structure will also oversee the Gender Action Plan. The aim is that each participant takes an active role in ensuring that awareness of gender issues is promoted and maintained (see 6.6).
8. DETAILED IMPLEMENTATION PLAN – FIRST 18 MONTHS

8.1 INTRODUCTION - GENERAL DESCRIPTION AND MILESTONES

The NeuroproMiSe Project is structured into four Scientific Subprojects:

Subproject Identification: Identification of candidate genes involved in inflammatory neurodegeneration
*Workpackages I1-I6*

Subproject Mechanisms: Analysis of key effector mechanisms of inflammatory CNS injury
*Workpackages M1-M4*

Subproject Validation: Development of new therapeutics for neuroprotection based on targets identified and validated in animal models
*Workpackages V1-V7*

Subproject Horizontal Integration: Integrated activities related to neuropathology, genomics and proteomics studies
*Workpackages H1-H2*

To achieve the common goal of developing novel therapeutics for inflammatory neurodegenerative diseases and the specific objectives defined for the first 18-month time period, the whole spectrum of methodological approaches available within the consortium will be utilized since the beginning of the project. While the WPs comprised in the Subproject ‘Identification’ will use genetic and functional genomic approaches to better define the role of known MS-related genes and to identify new genes and targets for therapeutic interventions, the WPs comprised in the Subprojects ‘Mechanisms’ and ‘Validation’ will focus on essential molecular and cellular pathways for neuroinflammation and neurodegeneration, and on the development, validation and/or optimisation of compounds with immunomodulatory and direct neuroprotective actions. With the exception of sodium channel blockers and TREM-2 modulating reagents, all the compounds to be tested within the first 18 months have been or are going to be developed by the consortium participants. The technological integration, to be achieved through the Subprojects ‘Horizontal Integration’ and ‘Training’, will ensure a tight conceptual and methodological linkage between the different WPs and will promote cooperative studies.

As evidenced in the PERT diagram (see below), the whole research programme is characterized by a profound interconnection (or circular flow) between the different subprojects, as the work concept includes both bottom up (starting from gene identification and validation, to drug development) and top down (from evaluation of the efficacy of already available or newly developed therapeutics to definition of underlying molecular mechanisms) approaches. This implies that most of the activities to be performed within each WP will extend beyond the initial 18-month period.

The specific objectives and milestones for the first 18 months are the following:

1) To identify major genes regulating the susceptibility to experimental inflammatory degeneration using a strategy based on selective recombinational selection in mice and rat congenic strains (Subproject Identification). This involves selection of minimized congenic strains that will allow to identify genes harbouring polymorphic effects on EAE development and neurodegeneration (month 18), and of partial advanced intercross lines for the study of gene-gene and gene-environment interactions (month 12 or 18). Once genes regulating or predisposing for disease are identified, further
studies will be performed to validate their role and dissect the underlying pathogenic pathways (> 18 months).

2) To set up the data-set and data-base structure (month 9) and complete genotyping and gene-gene interaction analysis of already identified genetic risk factors in MS (month 18) (Subproject Identification). These steps are essential to create a unique platform for subsequent evaluation of new candidate risk genes identified in the animal studies.

3) To define changes in global gene and protein expression profiles in different animal models of neurodegeneration (Subproject Identification). Gene and protein expression profiles and fully analyzed data-sets will be obtained by month 12-18, leading to the identification of genes and pathways involved in neuronal degeneration. This will set the basis for subsequent studies to validate the role of candidate genes and to evaluate whether common pathways of neurodegeneration are triggered in different model systems and might be targeted by therapeutic intervention.

4) To generate new experimental models and tools for the analysis of key effector mechanisms of inflammatory CNS injury and testing of new immunomodulatory strategies (Subproject Mechanisms). Doxycycline-inducible and humanized double transgenic mice will be generated to achieve new information on the role of autoreactive cytotoxic CD8+ T cells in CNS tissue damage (month 12/18). Triple transgenic mice expressing different human MHC class II molecules and human TCRs and a number of tools (soluble MS-associated MHC molecules and TCRs, DR15-MBP tetramers) will be generated (month 18) will allow to investigate further the disease modifying effect of epistatic interactions between MHC class II molecules and the disease modifying effect of the above compounds. Lentiviral vectors expressing molecules involved in the regulation of humoral immunity will be generated (month 12) and used to create new animal models to study the pathogenic role of lymphoid neogenesis in the inflamed CNS (month 18). In vitro culture systems will be used to acquire new knowledge on the signalling pathways involved in axonal dysfunction upon interaction with activated microglial cells (month 18). The above models will provide novel information on immunopathological pathways involved in CNS inflammation and will be used in subsequent studies aimed at evaluating the therapeutic efficacy of compounds specifically targeting such pathways and of therapeutics developed in other subprojects.

5) To test already existing and newly developed therapeutics for neuroprotection (Subproject Validation). To reach this goal, different tasks will run simultaneously and will involve: the development (month 6 or 9) and evaluation of the therapeutic efficacy (18 months) of compounds affecting TREM-2 and Ncf-1 pathways; the development of tools for selective stimulation of TNF receptor subtypes and of humanized transgenic mice for testing of such compounds (month 18); the investigation of TNFR1-mediated neuroprotective intracellular pathways (month 12) followed by the development of cell-penetrating small molecule drugs with neuroprotective activity (month 18); the evaluation of the neuroprotective efficacy of sodium channel blocking agents, inhibitors of the sodium-calcium exchanger, glutamate receptor antagonists and calcium channel blockers in in vitro and in vivo models (month 18); the development of cell-penetrating compounds with anti-apoptotic activity (month 6) and testing of their neuroprotective activity, either alone or in combination with immunomodulatory compounds (month 18). Collectively, the tools and knowledge generated by the above studies will allow to investigate further the activity and mechanism of action of candidate compounds, to identify those that are most effective in neuroprotection, and to develop new strategies for combined neuroprotective/immunomodulatory therapies in different animal models available within the consortium.

6) To set up reference centers, open to all consortium participants, for neuropathological, genomic and proteomic studies (Subproject Horizontal Integration) (month 7). These centers will be operating until the end, and possibly beyond, the project duration and will provide the appropriate coordination, know-how and technical support for the specific activities. Organization of genome-wide information collected from heterogeneous animal models into an artificial neural network (month 18) will represent an invaluable tool for evaluation and comparison of data sets derived from different studies.
as well as for assessing the outcome of experimental therapeutic regimens in subsequent stages of the project.

7) To set up an educational training programme to promote the synergies of the cooperating partners and to organize workshops to spread knowledge (including knowledge generated within the IP) within and outside the consortium. To this purpose, a programme of technical courses and meetings, personnel exchange, and focussed workshops will be prepared and disseminated through the website (month 10).

8) To set up the structure for coordination of Science, Business and Management of Neuropromise (month 1)

8.2 PLANNING AND TIME TABLE

Subproject Identification: ‘Identification of candidate genes involved in inflammatory neurodegeneration’
Co-ordinator: P3
Co-coordinator: P8
Participants: P2b, P4, P5, P6, P7, P9, P10, P17, P19

This Subproject has two main tasks. The first one is to identify major genes regulating the susceptibility to MS and experimental inflammatory neurodegeneration. The second one is to define changes in global gene and protein expression profile occurring during neurodegeneration in animal models. The two strategies complement each other as data derived from the genetic and the unbiased genome/proteome screening approaches can be integrated and help to focus on main candidate genes and molecular pathways involved in the pathological process underlying neurodegeneration (both inflammation- and non-inflammation driven). Validation of relevant genes and molecular pathways is another important task of this subproject which will be achieved through further studies of gene expression and localization and functional studies involving testing in appropriate in vitro and in vivo systems as well as generation of new animal models (transgenic mice), these activities extending beyond the 18-month period.

Task 1: During the initial 18-month period, the use of the animal platform established by P3 and P8 will lead to further positioning and isolation of genes within already identified loci regulating EAE development and neurodegeneration in mice and rats. Genetic studies in humans will be also initiated to obtain new genotypic information in the context of already known genes and to control for epistasis and gene-gene interactions.

WP I1: Mouse genetic platform for the identification of neuroinflammatory disease-related genes. This WP starts at month 1, involves P3 and P17 and aims to identify and validate genes regulating EAE in mice. The strategy to isolate the genes is based on selective recombinational selection in congenic strains. This analysis will take advantage from the fact that the major loci containing genes controlling chronic relapsing EAE have already been mapped and isolated in well defined congenic strains by P3. Fifteen different mouse congenic strains are available and will be used for positional cloning of the genes in these loci through minimizing the congenic fragment, and for developing and analysing gene interactions in partial advanced intercross lines. Gene expression pattern within the selected congenics will be analysed and the responsible polymorphism will be determined by sequence analysis. It is expected that within the 18-month period, 5 minimized congenic mouse strains, suitable for positional cloning of the underlying polymorphic genes, and one partial advanced intercross line will be isolated and characterized. At least one positionally cloned gene with polymorphic effects on EAE development will be identified. For this gene, functional studies will be started (including
immunological studies) in collaboration with P17, and knock-in or knock-out mouse lines will be generated; these latter activities will extend beyond 18 months.

WP I2: Rat genetic platform for the identification of neuroinflammatory disease-related genes. This WP starts at month 1, involves mainly P8 in collaboration with P3 and P5, and aims to define and validate genes involved in EAE and neurodegeneration in rats. Also for this species the most important loci regulating EAE have been isolated in congenic strains and the strategy utilized to identify gene polymorphisms and gene-gene interactions relevant to disease is similar to that described in WP I1. However, in addition to EAE-regulating genes, this WP will also focus on neurodegeneration used subtraits, by utilizing a model of neurodegeneration and local activation of CNS innate immunity induced by ventral nerve root avulsion, already established by P8 group. The loci controlling neurodegeneration and inflammation in this model have already been fine-mapped in an advanced intercross line (AIL) between the DA and PVG strains, and further recombinational selection of congenic strains, gene sequencing, haplotype analysis and expression analysis, to be performed within the 18-month period in collaboration with P3, will allow exact gene positioning and definition of the molecular pathways involved. It is expected that within this period at least 5 minimized congenic rat strains containing less than 10 genes regulating EAE and one partial advanced intercross line for the study of gene interactions will be established. Furthermore, at least two new genes harbouring polymorphic effects on EAE development and neurodegeneration in the rat will be positionally cloned. The influence of a functional polymorphism in the CIITA gene controlling MHC class II expression and in the Ncf-1 gene, identified in previous studies by P3, will be further examined using rat strains congenic for the corresponding genome region. Direct studies of gene-gene interactions will be done after breeding of bicongenic strains (CIITA-Ncf-1) to study the combined role of polymorphisms in these two genes for neuroinflammatory disease and attention will be given to the function of antigen presenting cells. Further gene-gene interactions will be studied by systematic combination with the other congenic lines at hand in the consortium. Unbiased gene expression studies of material from the CIITA and Ncf-1 congenic lines and parental strains will enable deciphering of the molecular pathways affected by the polymorphic genes. For the CIITA gene, a wide variety of neuroinflammatory model diseases, such as different EAE models and neuroinfections, will be titrated and applied to the congenic and parental strains, to systematically establish how an evolutionary conserved polymorphism in the CIITA gene, leading to strikingly different expression of MHC molecules, regulates/predisposes for disease. As far as possible, the same assays developed experimentally will be taken in a translational approach to materials from humans having similar differing genotypes with consequences for MHC class I and II expression. Hereby, already with the established gene polymorphisms, there is basis for examining the possible use of CIITA polymorphisms as a pharmacogenetic marker in large cohorts of patients with MS. Functional ex vivo assays may add to this. Detailed neuropathological analysis of congenic and AIL lines will be performed in collaboration with P5.

WP I3: Identification of susceptibility genes in MS patients This WP starts at month 1 and involves P3, P8 and P9. The first step is to create the data-set and the data-base. Most samples are already available, as either blood or DNA but need to be made useful by DNA preparation, concentration measurement, clinical validation and dispensing in 96-well plates. A locally developed database for DNA storage is available by P8 and will be shared with P9. Clinical information likewise needs to be data-based. Then, an integration of the banking and clinical databases will be performed. Finally, a database system to store genotyping information is likewise being adapted at KI (P8) and will be shared with P9. We propose that parallel rather than joint database systems will be set up in order to cope with national legislations on handling of genetic information. This work will be performed during the first 9 months of the project, which is likely, since such efforts have already been initiated. The second step will be genotyping of all persons within the datasets with regard to already identified genetic risk factors in MS (HLA class II, class I, PRKCA, CIITA and CTLA-4). Genotype information regarding HLA class II is available in both centres, whereas PRKCA is available only for Finnish patients and HLA class I, CIITA and CTLA-4 mostly for Swedish patients. P3 will analyse in detail the structure of the Ncf1 gene, which is duplicated due to a human specific duplication event and with variable numbers of copies of both pseudo, allelic and normal genes. The goal will be to develop assays for genotyping and typing of copy numbers. These tasks will be completed by month 9.
From month 9 to month 18, a gene-gene interaction analysis of the basic genotypes will be performed. Here, present methods are partly insufficient and development of practical and statistically acceptable methods is required. P9 has previously reported such methods and P8 is presently engaged in the development of such procedures. Therefore, it is proposed that this task will be completed within 9 months, thereby enabling a similar analysis for each additional gene being identified during the project.

Thus, starting from month 18, the participants involved in this WP will be prepared to test new genes for a role in MS susceptibility in a streamlined system enabling the analysis of patient stratification and gene-gene interaction. P5 will analyse the Ncf1 gene in larger material and will also make haplotype analysis of the remaining Ncf1 pathway genes, in cooperation with P8 and P9.

Task 2: During the initial 18-month period, the activities of the three following WPs will concern the analysis of the gene and protein expression changes associated with neurodegeneration in three different animal models, each mimicking particular aspects of the pathological changes observed in MS lesions and other neuroinflammatory diseases (namely, altered myelin-axonal interactions, dysregulated microglia activation, and axonal transection).

WP I4: Genomics of axonal degeneration in myelin mutant mice. This WP will examine gene expression changes in well defined neuronal and glial cell populations during neurodegeneration in myelin mutants. It starts at month 1 and involves mainly P7 in cooperation with P2b for immunohistochemical studies and P2 for gene validation analysis. P7 has established and phenotypically characterized mouse lines with mutations in myelin-specific genes (CNP, PLP), which undergo neurodegeneration in the absence of evident demyelination. These mice represent a powerful tool to examine neurodegenerative changes in a situation which reproduces altered myelin-axon interactions and is likely to mimic, at least in part, the situation in MS demyelinated plaques. During the first 18 months, the procedure for transgenic labelling of cell nuclei and isolation of defined neuronal and oligodendrocyte cell populations in tissue sections from different CNS areas by fluorescence-directed laser microdissection will be established. This procedure will be applied at both early and advanced stages of neurodegeneration. Groups of as few as 50 identifiable cells will be isolated and collected in appropriate tubes containing mRNA extraction solutions. Global transcriptome analysis will be performed using Affymetrix whole genome microarrays. Different myelin mutant mice with similar neurodegenerative phenotypes will be analysed to cross-control the microarray results. It is expected that by the end of the 18-month period, gene expression profiles (raw data) of defined cortical and cerebellar neuronal populations and of corresponding white matter oligodendrocytes will be obtained and the data set will be analysed. For gene validation, quantitative real-time PCR, in situ hybridization and immunohistochemistry will be performed in mutant mice at distinct time points. These latter activities will extend beyond the 18-month period.

WP I5: Genomics of inflammatory neurodegeneration in DAP12 and TREM-2 mutant mice. This WP will examine changes in gene transcription in DAP12 and TREM-2 mutant mice made available by P9 and P19. Gene expression profiling will be performed by P9 in DAP12 and TREM-2 mutant mice at early and late disease stages to identify new genes/molecules involved in synaptic dysfunction (early disease stage) and inflammatory neurodegeneration (late disease stage). RNA will be isolated from distinct brain regions at defined age of mutant and control mice. Labeled RNA will be hybridized to Affymetrix whole genome microarrays. It is expected that by the end of the 18-month period, gene expression profiles (raw data) of control and DAP-12/TREM-2 mutant mice will be obtained and the data set will be analysed. Validation of genes involved in inflammatory neurodegeneration will be performed by P19, by quantitative real-time PCR and in situ hybridization in normal mice and in mutant mice at distinct time points. These latter activities will extend beyond the 18-month period.

WP I6: Proteomics of axonal degeneration in a model of optic nerve transection. This WP starts at month 1 and involves P10. The proteome association with axons undergoing Wallerian degeneration in the CNS will be examined. The proteomic profile will be carried out on optic nerves at selected time points after axon transection considering an early time point, 12 hours, and a later time point, 48 hours, initially. Material will be harvested with special attention paid to the removal of the site of injury and the meningeal tissue since they will add noise to the system. Initial studies will be...
performed on normal nerves to enrich for the axon membrane and axoplasm with the aim of reducing the amount of material that is derived from the myelin sheath and the glial cell populations, astrocytes oligodendrocytes and microglia. Different extraction protocols and differential centrifugation will be performed to optimise the yield of axon derived material as confirmed by Western blotting. Preliminary studies using modifications of protocols derived from the literature indicate that significant enrichment of axon-derived material is readily possible. This material will then be treated according to the ICAT protocol and mass-spectrometry analysis. It is expected that by the end of the first 18 months initial proteomic profiles of normal axon derived material and a profile for axons undergoing degeneration at two selected time points will be made available. Immunohistochemistry will be used to visualise expression patterns of molecules for which there are available antibodies. Further data analysis and analysis of proteins of interest will extend beyond this time, with comparison and complementation of the ongoing genetic studies.

Subproject Mechanisms: Analysis of key effector mechanisms of inflammatory CNS injury

Coordinator: P1
Co-coordinator: P6
Participants: P2a, P2b, P3, P4, P5, P10, P19

WP M1: CD8+-T-cell-mediated neurotoxicity in humanized animal models. This WP starts at month 1 and involves mainly P6 for the generation of transgenic mice, in collaboration with P5 for the neuropathological evaluation and P2a for analysis of specific mechanisms of neurodegeneration. A transgenic mouse system which comprises two components will be established: 1) already available lines of transgenic mice expressing a T-cell receptor (TCR) specific for the hemagglutinin (HA) protein of the influenza virus on either CD8+ or CD4+ T-cells; and 2) to be generated transgenic mice expressing HA as a neo-self-antigen in CNS glial cells or neurons. To generate HA-expressing mice, two independent strategies will be adopted: (i) the targeting of the Rosa26 locus by homologous recombination, and (ii) the development by classical transgenesis of an inducible model of HA expression by the use of the tetracycline inducible system. The Rosa26 locus will be targeted to generate mice in which the HA gene can be switched on in any cell type, using a strategy based on the conditional removal of a transcriptional STOP cassette by the CRE recombinase. Several mice expressing the CRE protein in specific CNS cell populations (oligodendrocytes, neurons, astrocytes) have been generated (www.mshri.on.ca/nagy/). In particular, P6 is currently breeding the MOG-CRE line that mediates a highly specific CRE-mediated recombination in oligodendrocytes. The Rosa26 HA knock-in mice will be obtained by month 3. Thereafter, these Rosa26 HA knock-in animals will initially be crossed with MOG-CRE mice to induce specific HA expression in oligodendrocytes (month 8). Subsequently, the transfer of graded numbers of HA-specific cytotoxic CD8+ T cells (obtained from the HA-specific CD8 TCR transgenic mice) will allow to assess the detrimental potential of CNS-infiltrating CD8+ T lymphocytes targeting the myelinating cells (month 12). The clinical, neuropathological and immunological features of these CD8+ T-cell-mediated models of CNS autoimmunity will be examined and will be compared to models of neuroinflammation induced by HA-specific CD4+ T cells already available in the laboratory (month 18). Following a similar approach, we will then establish new transgenic mouse models for the study of CD8+-mediated neuronal/axonal cell damage by crossing the Rosa26 HA knock-in animals with mice expressing CRE in neurons (CamKIIalpha-CRE, GluRdelta2-CRE) (> month 18).

The HA inducible transgenic model will be generated by micro-injection of a transgene consisting of a bidirectional inducible promoter governing the expression of both HA and the LacZ reporter gene. Several transgenic lines will be generated, and crossed with already available CamKIIalpha-rtTA transgenic mice that express the rtTA transactivator in CNS neuronal populations (month 9). After administration of doxycycline to the double transgenic animals, the expression of both transgenes will be assessed by RT-PCR, Western blotting and X-Gal staining of fresh brain sections (month 18). With this model, it will be possible to dissociate the resulting CNS resident cell damage from the harmful T-cell inflammation by turning off CNS HA expression.
WP M2: Epistatic interactions in the MS-associated DR2 haplotype in humanized mouse models. Within the first 18-month period, this WP will verify whether an epistatic interaction between the DR15 and 51 molecules will lead to a modified disease course in mice expressing both these MHC class molecules. This WP starts at month 1 and involves mainly P4. P2b and P3 will cooperate for neuropathological and immunological studies, respectively. The initial activities will involve expansion of the colony of transgenic mice expressing the MS-associated MS MHC class II molecules DR15 and DR51 and an MS patient-derived MS T cell receptor recognizing MBP and virus-derived peptides. These mice will also be backcrossed to the Rag-/- background to get mice in which the only expressed T cell receptor is the one encoded by the transgenes. Mice will be examined for spontaneous development of MS-like disease, by evaluation of clinical, immunological and neuropathological parameters. Disease development and course will be also examined following immunization with MBP and viral peptides. DR15-MBP tetramers generated by P6 group will be used to quantify and characterize autoreactive DR15-MBP specific T cells in mice that express either DR15 and/or DR51. Ex vivo studies will examine potential differences in signalling pathways in cross-reactive T cells to understand the functional basis for how different stimuli from either MBP or viral peptides lead to different disease patterns. To further characterize the biophysical basis for T-cell receptor cross-reactivity, soluble T cell receptors will be expressed in E. coli and their binding affinity and kinetics in their interaction with soluble MHC class II-epitope complexes will be characterized by using surface plasmon resonance (Biacore) measurements. Furthermore, experiments will be initiated to verify the disease modifying effect of blocking disease-associated MHC class II molecules with a mAb recognizing the HLA-DR15-MBP 85-99 complex or targeting autoreactive T cells with DR15-MBP tetramers. The data obtained within the first 18-month period will be utilized for further investigations on disease modifying interactions between MHC class I and class II genes to be performed at later times.

WP M3: Intracerebral lymphoid tissue in CNS tissue injury. This WP starts at month 1 and involves mainly P1, in collaboration with P2b for neuropathological studies in MS tissue and P6 for immunological studies (analysis of autoimmune T and B cell responses). Two specific tasks will be executed. The first one deals with the immunohistochemical screening of a large number of autoptic brain tissue samples from patients with different MS courses (obtained form P2b, P5 and the UK Multiple Sclerosis Tissue Bank), to search for intrameningeal lymphoid structures (or B-cell follicles) and correlate their presence with the extent of CNS tissue destruction (both gray and white matter demyelination, axonal damage) and inflammation. Through this screening, it is expected that within 18 months new information will be generated on the pathogenic relevance of abnormal lymphoid neogenesis in the autoimmune CNS. When available, blocks of snap frozen tissue from brain tissue with lymphoid follicles will be selected and, using a laser microdissector, will be collected in appropriate tubes containing mRNA extraction solutions and used for examining gene expression of molecules involved in lymphoid neogenesis.

The second task deals with the construction of lentiviral vectors for the transduction of two molecules involved in B-cell responses and lymphoid neogenesis, namely BAFF and CXCL13. The aim here is to verify whether overexpression of BAFF or CXCL13 in the CNS can lead to spontaneous or exaggerated EAE-induced formation of lymphoid follicles in the meninges. For an optimal transduction efficiency, the lentiviral particles will be pseudotyped with the Vesicular Stomatitis Virus Glycoprotein (VSV-G), a viral receptor recognizing a ubiquitous cell receptor, thus allowing efficient transduction in virtually all cell types. Transducing lentiviral vector (LV) particles will be recovered by means of transient co-transfection of 293T cells with a lentiviral packaging construct together with “third generation” (i.e.CMV-promoted lentiviral vectors expressing the respective transgenes) and VSV-G expressing vectors. The efficiency of LV particle production will be assessed by the quantification of the HIV-1 Gag p24 content using commercial ELISA kits and reverse transcriptase assay. Finally, the transducing titers of LV preparations will be assessed by the in vitro infection of at least three murine cell lines with serially dilutions of each viral preparation. Each lentiviral vector will be injected into the cisterna magna of SJL mice and transgene expression as well as CNS alterations (if any) will be monitored at different times using RT-PCR and immunohistochemical techniques. The consequences of BAFF and CXCL13 overexpression in the meningeal/ventricular compartment will be also evaluated following immunization with PLP 139-151 and induction of EAE, through
assessment of clinical, neuropathological and immunological parameters. These latter studies will extend over the initial 18-month period.

WP M4: Mechanisms and key intracellular regulators of microglia-mediated inflammatory axonal injury. This WP starts at month 1 and involves mainly P19, in collaboration with P2a for analysis of intracellular pathways involved in axonal damage and P2b for immunocytochemical studies. During the first 18-month period, the molecular mechanisms of microglial-mediated neuronal/axonal injury will be analyzed in *in vitro* systems which will require the establishment of primary neuronal, mixed microglial-neuronal and organotypic brain tissue cultures from the murine CNS. Cultured neurons will be transfected with neuronal promoter specific GFP-tagged synaptic genes, such as synaptophysin and beta-amyloid precursor protein or mitochondrial genes to visualize axonal transport. Prestimulated microglial cells or their inflammatory mediators (TNF, nitric oxide) will be added to the transfected neurons. Effects of inflammation on axonal transport and synaptic integrity will be monitored by time lapse microscopy and confocal/2-photon laser scanning microscopy. Possible structural axonal cytoskeleton changes will be detected by immunostaining with specific fluorescent tagged antibodies directed against tau, tubulin and neurofilament after fixation of the cells. The signaling pathways induced by microglial inflammatory mediators resulting in axonal dysfunction and injury will be studied in these *in vitro* cultures by small molecule inhibitors. Particularly, involvement of caspase and stress kinase (JNK) signaling pathways in axonal transport disturbance and injury will be analyzed using kinase inhibitors. These studies, which should be completed by month 18, will provide a new model system as well as new knowledge on inflammation-induced axonal injury which will be useful for evaluation of neuroprotective strategies based on newly identified genes or on compounds available through the consortium.

Subproject Validation: Development of new therapeutics for neuroprotection based on targets identified and validated in animal models

**Coordinator:** P19  
**Co-coordinator:** P11  
**Participants:** P1, P2a, P2b, P3, P5, P6, P12, P13, P14, P16, P17, P18, P20

WP V1: New therapeutics targeting the TREM-2 pathway. This WP starts at month 7 and involves mainly P19, in collaboration with P1 for studies in brain cell cultures and EAE, and P2b for neuropathological analysis and TREM-2 localization in EAE- and MS-afflicted brain tissue. The tools necessary to study TREM-2 localization and function in the CNS will be generated and provided by Bioxell (Milan, Italy) (utilization of this material by P1 and P19, and at a later time possibly by other partners, will be regulated by a Material Transfer and Research Agreement). The TREM-2 reagents include: anti-human and mouse TREM-2 mAbs (with agonistic activity); soluble mouse and human TREM-2/IgG fusion proteins; TREM-2 small molecular weight antagonists; and a TREM-2/DAP-12 reporter cell line useful for the characterization of TREM-2 ligands. By month 7, experiments will be started to examine the effects of TREM-2 mAbs and fusion proteins on basal and stimulated functions (antigen presentation, cytokine/chemokine production, NO and PGE2 release, phagocytosis, axonal damage) of cultured murine microglia (P19). Co-cultures of microglia and astrocytes (a likely source of TREM-2 ligand(s)) will be also established to evaluate the involvement of the TREM-2/TREM-2 ligand(s) pathway in cell-cell interactions which were previously shown to affect microglial pro-inflammatory activities (P1). By month 12, experiments will be initiated to test the influence of TREM-2-modulating reagents (specifically, activating anti-TREM-2 mab and TREM-2-Ig fusion proteins) in different EAE models (MOG 35-55-induced, chronic EAE in C57BL/6 mice and PLP 139-151-induced, relapsing-remitting EAE in SJL mice). Both the anti-human and anti-mouse TREM-2 antibodies will be tested in control and in MS and EAE brain/spinal cord tissues using immunohistochemical techniques to examine the distribution of TREM-2 during the course of CNS inflammation. These two latter sets of experiments will extend beyond 18 months.

WP V2: New therapeutics targeting the Ncf1 pathway. This WP starts at month 1 and has two major tasks: the first one is to shed light into the molecular pathways regulated by *Ncf-1*, a susceptibility
gene for experimental arthritis and EAE; the second task is to identify a novel Ncf1 agonist that specifically activates ROS production from the NADPH oxidase complex in a tissue- and time-specific manner. This agent will be developed by P17, a biotechnology company, in collaboration with P3. The initial work will be aimed to understand the impact of the Ncf1/NADPH complex on the immune response, in particular on antigen presentation and costimulation. Initial experiments will address the behaviour of T cells and APCs isolated from collagen II specific TCR Vβ12 transgenic mice combined with Ncf1 mutated mice; in a second step, T cells and APCs will be isolated from mice carrying a Ncf1 mutation and backcrossed into an EAE-susceptible mouse strain. The influence of the Ncf1 mutation on T-cell and APC function will be examined using several methods, including microarray analysis followed by real time PCR and proteomic analysis, to identify all differentially expressed products between wild type and Ncf1 mutated mice. The definition of genetic profiles linked to the Ncf1 pathway will be achieved within the first 18 months of the project and is a necessary prerequisite for subsequent evaluation of the distribution, efficacy and safety of potential drug candidates targeting the Ncf-1 pathway(s). In parallel, experiments will be started to test the efficacy of new chemical structures developed by P17 in enhancing the activity of NAPDH oxidase in in vitro systems and in modulating EAE. These latter activities will extend beyond 18 months.

WP V3: New therapeutics selective for TNF receptor subtypes and humanized animal models. This WP starts at month 1, involves mainly P12, P13 and P16, in collaboration with P1 for the in vitro and EAE studies. This WP has 3 specific tasks: 1) to develop human TNFR specific reagents (P12); 2) to develop mouse TNFR specific reagents (P16); and 3) to generate humanized TNFR mice for evaluation of the therapeutic efficacy of human reagents (P13). For selective activation of human TNFR2, two strategies will be used. The first strategy, involves covalent coupling of an already generated human TNFR2 specific, genetically stabilized TNF mutein (trimer encoded as a single chain protein with trimeric TNF units connected via a flexible linker) with nanocarrier particles to enhance its specific bioactivity and promote brain delivery (optimization of particle properties and characterization of particles by P12 in collaboration with P20; proof of principle has been already given). The second strategy makes use or a TNFR2-selective single chain TNF mutein (P12; available) which will be fused to single chain antibodies (hybridomas O1 and O4 from P1, cloning by P12 in collaboration with P20) directed towards oligodendrocyte-specific markers. These reagents will be characterized with respect to their expression in appropriate hosts, binding properties, specificity, in-vitro and in-vivo stability and toxicity in collaboration with P20. For the development of human tools, an already available TNFR1 specific antibody (H398, developed by P12) will be used. This antibody will be expressed as a scFv derivative and suitable pharmacological formulations for increased stability (e.g. PEGylation, liposomal encapsulation) will be tested initially in in vitro models (TNF antagonistic activity and stability) and later in humanized TNFR1 mice in appropriate MS models.

Monoclonal antibodies with antagonistic activity for murine TNFR1 and agonistic activity for TNFR2 will be developed by P16 and will be available between month 12 and 18. To test the activity of these antibodies in protecting neural cells from toxic insults, starting from month 12 neuronal and oligodendrocyte cultures will be established from the fetal and neonatal mouse brain, respectively, and exposed to an excitotoxic stimulus, both in the absence and presence of the TNFR specific antibodies. Depending on the in vitro results, TNFR agonists and antagonists will be also tested in vivo for their neuroprotective activity using both models of acute inflammation (ischemia) (P13) and of chronic and relapsing EAE (P1). Both the in vitro and in vivo studies will extend beyond 18 months.

P13 will generate humanized TNFR1 and TNFR2 mouse lines by knock-in strategies; these lines will be used in subsequent studies (beyond 18 months) to investigate the neuroprotective efficacy of human TNFR specific compounds in in vitro and in vivo models (ischemia, EAE, excitotoxic injury). In the first 18-month period, embryonic stem cells (R1 and RW4) will be electroporated with targeting constructs consisting of large parts of the mouse TNFR1 and TNFR2 gene locus, respectively. For positive/negative selection, a floxed neo cassette downstream of the cDNA cassette and a terminally located herpes simplex virus TK cassette will used. Stable clones with homologous recombination of each construct will then be isolated and injected to generate chimeric mice and breed for founder animals.
WP V5: Axonal protection achieved by blockers of the sodium channel and the sodium/calcium exchanger. This WP starts at month 1 and involves mainly P14, in collaboration with P5 for histological analysis of neuronal degeneration and P12 for combination therapies with TNFR-specific agnosis and antagonists. This WP has 2 specific tasks: 1) to validate the role of the neuronal TNFR1 core signaling scaffold, and key intracellular signaling components in neuroprotection; and 2) to rationally design and develop cell-penetrating small molecule drugs based upon this mechanism that will enhance neuron (and possibly axon) protection in vivo. Following recent evidence that classical apoptotic mechanisms contribute to neuron death under conditions of ischemic injury (e.g. see success of pre-clinical studies with small JNK inhibiting peptides; Borsello et al., 2003, Nature Med. 9:1180(P14, publication under revision), and that neuronal TNFR1 signaling is necessary for neuroprotection through a caspase-dependent mechanism (P14, publication under revision), the contribution of apical signalling components of death receptor signalling pathways to neuron death/survival decisions needs to be validated. Two strategies will be followed. First, siRNA for caspase 8 and the long isoform of FLIP (FLIPL), likely the major regulators of death receptor-induced apoptosis, will be delivered to cultured primary neurons using lentiviral vectors and the consequences of gene knockdown will be measured under conditions of ischemic neuronal injury (glucose deprivation and oxygen/glucose deprivation) and apoptosis induction (chemically- or ligand-mediated). Second, mutant forms of caspase 8, FLIPL, as well as the adaptor protein FADD and RIP, will be fused to the TAT sequence of HIV for efficient intracellular delivery and overexpression in primary neurons. Mutants will include dominant negative, protease-deficient and uncleavable/precleaved forms of these proteins (P14: available). The neuroprotective versus neurotoxic effects of these cell-penetrating peptides will be measured in vitro as described above. Using the information gained, we will go on to rationally design and develop small neuroprotective TAT-fused molecules and test their efficacy for protecting neurons in vitro, and neurons/axons in vivo using experimental models for MS (MOG-EAE) and stroke (middle cerebral artery occlusion) (within 18 months), and further develop these (beyond 18 months) for neuron-targeted delivery and pre-clinical assessment in vivo.

WP V4: Small drug molecules targeting the neuronal TNFR1 core signaling scaffold. This WP starts at month 1 and involves mainly P14, in collaboration with P5 for histological analysis of neuronal degeneration and P12 for combination therapies with TNFR-specific agnosis and antagonists. This WP has 2 specific tasks: 1) to validate the role of the neuronal TNFR1 core signaling scaffold, and key intracellular signaling components in neuroprotection; and 2) to rationally design and develop cell-penetrating small molecule drugs based upon this mechanism that will enhance neuron (and possibly axon) protection in vivo. Following recent evidence that classical apoptotic mechanisms contribute to neuron death under conditions of ischemic injury (e.g. see success of pre-clinical studies with small JNK inhibiting peptides; Borsello et al., 2003, Nature Med. 9:1180(P14, publication under revision), and that neuronal TNFR1 signaling is necessary for neuroprotection through a caspase-dependent mechanism (P14, publication under revision), the contribution of apical signalling components of death receptor signalling pathways to neuron death/survival decisions needs to be validated. Two strategies will be followed. First, siRNA for caspase 8 and the long isoform of FLIP (FLIPL), likely the major regulators of death receptor-induced apoptosis, will be delivered to cultured primary neurons using lentiviral vectors and the consequences of gene knockdown will be measured under conditions of ischemic neuronal injury (glucose deprivation and oxygen/glucose deprivation) and apoptosis induction (chemically- or ligand-mediated). Second, mutant forms of caspase 8, FLIPL, as well as the adaptor protein FADD and RIP, will be fused to the TAT sequence of HIV for efficient intracellular delivery and overexpression in primary neurons. Mutants will include dominant negative, protease-deficient and uncleavable/precleaved forms of these proteins (P14: available). The neuroprotective versus neurotoxic effects of these cell-penetrating peptides will be measured in vitro as described above. Using the information gained, we will go on to rationally design and develop small neuroprotective TAT-fused molecules and test their efficacy for protecting neurons in vitro, and neurons/axons in vivo using experimental models for MS (MOG-EAE) and stroke (middle cerebral artery occlusion) (within 18 months), and further develop these (beyond 18 months) for neuron-targeted delivery and pre-clinical assessment in vivo.

WP V5: Axonal protection achieved by blockers of the sodium channel and the sodium/calcium exchanger. This WP starts at month 1 and aims to identify the optimal drugs for protecting axons from degeneration due to inflammatory demyelinating disease. It involves mainly P11, in collaboration with P5 for histological evaluation of signs of axonal damage. During the first 18 month period, two therapeutic strategies will be examined: first the partial blockade of sodium channels, and second the blockade of the reverse mode of action of the sodium calcium exchanger. Preliminary data from P11 indicate that both strategies will be effective, but that the best agents have to be identified. In a first step, simpler experimental models will be employed to identify agents that are likely to be effective in the more sophisticated trials. Sodium channel blocking agents have been widely applied in patients for a number of disorders and so it is fortunate for the proposed research that there is now a range of agents available, and several of these are at least partially characterised for their biophysical effects. Thus, some agents may, for example, block the open sodium channel, while others may bind to the inactivated channel. The availability of drugs is therefore not a problem, and several of the drugs are known to be clinically safe. However, detailed knowledge of which drugs preferentially affect different sub-types of sodium channel, when operating in different modes (e.g. persistent vs. transient sodium current), is not known. It is also true that even if this knowledge was available, the information would be of limited use as we remain uncertain of which sub-types of channel, operating in which mode, are important in causing axonal degeneration. Thus, it is not yet possible to make rational predictions about the efficacy of the available drugs. Therefore, a panel of drugs having a variety of properties will be tested, to try to assess which features of the drugs recommend them for axonal protection. Some of the drugs will be novel, arising from links with the pharmaceutical industry (outside this consortium). The initial screen will involve excised rat spinal roots, and it is possible to obtain sufficient tissue from one animal to screen about 5 drugs, each examined over a range of 4 different concentrations. The drugs will be applied to roots in vitro, with the roots exposed for 2 hours to an axonally damaging concentration of nitric oxide. The roots will then be examined histologically to determine if any drugs are effective in axonal protection (integrity of axolemma, microtubules, neurofilaments etc). Other, similar experiments will assess the efficacy of blockers of the sodium/calcium exchanger. Only a few such drugs have been identified (e.g. bepridil) and so the available choice will be limited, although some novel drugs with the appropriate properties can be
provided by our industrial contacts. It is expected that the above activities will be completed by month 18; the following step will be to validate the choice of agents by demonstrating their efficacy in models of EAE (after 18 months).

**WP V6: Axonal protection by glutamate receptor and calcium channel blocking compounds.** This WP starts at month 1 and involves mainly a SME, P18, in collaboration with P11. P18 has demonstrated the value of glutamate (AMPA) receptor blockade for axonal protection in EAE, and has developed a range of novel pharmaceutical agents that show promise in initial experiments and now need to be validated in detail using the models of axonal injury and the ex-vivo experimental approaches utilized by P11, as well as the existing models of EAE. The laboratories of P11 and P18 are nearby in London, and have complementary expertise, so that the successful funding of this application is expected to result in synergistic advances. P18 also has novel calcium channel blocking agents that will be shared with P11 to take advantage of the different experimental approaches available in P11's laboratory, in combination with techniques of calcium measurement. It is expected that by month 18, these activities will lead to the definition of the contribution of glutamate and calcium channel signaling in axonal degeneration and to the identification of the best pharmacological agents for use in neuroprotection in other neuroinflammatory and neurodegenerative model systems available in the consortium.

**WP V7: Combination therapies with neuroprotective anti-apoptotic and anti-inflammatory drugs.** This WP starts at month 1 and involves P2a in collaboration with P2b for histopathological analysis of rat optic nerves. P2a has established an optic neuritis model with strong neurodegenerative aspects in which a functional, electrophysiological monitoring of axons and neuronal cell bodies can be performed. By stereotactic injection of a fluorescent dye into both superior colliculi, retinal ganglion cells will be retrogradely labeled for later counting and their function will be monitored by recording of electroretinograms (beside the recording of visual evoked potentials for optic nerve function). In order to identify neuroprotective substances for further combination therapies (neuroprotective plus immunomodulatory/anti-inflammatory), two main therapeutic approaches will be tested during the first 18 month: First, minocycline, flupirtine, and simvastatin, three agents already approved for the treatment of humans, will be tested with respect to retinal ganglion cell as well as optic nerve axon fiber survival and function. Preliminary data of P2a show that these three substances can influence anti-apoptotic signaling pathways in neurons. They can be given orally and have good safety records which would allow fast transfer of the results into clinical trials. Second, molecules with defined anti-apoptotic properties (Bcl-2, GDNF) will be applied using the TAT protein as a carrier. Respective TAT fusion proteins have been developed by P2a and were successfully applied in an animal model of cerebral ischemia. By month 18, it is expected that neuroprotective agents will be identified, which can be combined with methylprednisolone and/or established immunomodulators and/or substances developed by other partners. The primary aim of these combination therapies is to obtain synergistic effects. However, combination with methylprednisolone can also be necessary to control potential unwanted side effects of those drugs leading to non-neuronal Bcl-2 induction (e.g., in immune cells). After single approaches have been shown to act neuroprotectively, they will be tested again using a more sophisticated model including MRI imaging of the optic nerves.

**Subproject ‘Horizontal Integration’: Integrated activities related to neuropathology, genomics and proteomics studies**

**Coordinator:** P5

**Participants:** P2b, P3, P9, P10, P14, P17

**WP H1: Neuropromise Neuropathology Reference Center (NNRC).** This WP starts at month 1 and involves P2b and P5. The two participants will set up a dedicated group within their respective laboratories to plan and execute neuropathological analyses of relevant tissues (MS tissue provided by P2b and P5, and animal tissues provided by the other participants) in strict collaboration with the consortium partners and in relation with the specific tasks addressed in the respective WPs.
Within the initial 18-month period, P2b and P5 will first characterize the lesions in autopsy and biopsy material from MS patients by quantitatively determining the extent of inflammation, demyelination, axonal injury and loss, as well as remyelination. In addition, the cellular composition of inflammatory infiltrates in different phases of the disease (acute, relapsing and progressive) will be determined by quantitative immunohistochemistry. This tissue material will then be available to study the expression of candidate molecules, identified in the Subprojects “Identification” and “Mechanisms”, within different types of multiple sclerosis lesions, and to determine their involvement in the pathogenesis of the disease process.

In parallel, DNA will be extracted from the paraffin blocks and stored for further genetic analysis, in collaboration with P8 and P9. From the extracted DNA, in a first step the HLA and Apo-E genotype of the patients will be determined. In a second step, which will extend over the first 18 months, polymorphisms of other candidate genes, which so far came out from genetic studies in MS patients will be analysed (e.g. NOS-2A, cytokine genes, CTLA-4, CNTF and others). The genotype will then be correlated with the pathological phenotype (extent and composition of inflammatory infiltrates, demyelination, axonal injury and remyelination).

In parallel to the human studies, brain and spinal cord tissue provided by other partners of the consortium will be analysed for inflammation, demyelination, axonal injury and remyelination, to determine the pathological phenotype in the respective experiments.

WP H2: The NeuroproMiSe Genomic and Proteomic Reference Centers. This WP starts at month 7 and involves P3, P9, P14, and P17 for microarray analysis and P10 for the proteomic analysis.

During the first 18 months, a major task of these participants will be to standardize as much as possible the data obtained within the consortium from gene expression studies to allow subsequent integration and optimisation of the results. This will be done by taking into consideration:

a) the genetic background of the mouse and rat strains used. For example, the C57BL/6 mouse strain, which is used by several participants (P2a, P3, P4, P6, P14), presents several advantages, like: i) compatibility with most experimental models utilized by the consortium; ii) immunologically is well characterized; iii) the genomic sequence is available; iv) ES cells are available for genetic studies; v) available gene expression data from P3 and P14 and in public data bases are from this strain;

b) age, sex, tissue specificity and duplicacy of samples;

c) documentation of disease timing (initiation, progression and resolution of disease);

d) choice of controls (including paired controls within each experiment, a single reference RNA for the whole consortium or comparison with a universal-available mouse or rat RNA). This will be decided within the consortium.

A plan will be established to collect material from the interested partners and provide, within the different centers, the necessary support for microarrays and proteomics, as well as for bioinformatic analysis.

Another major task of this WP will be the construction of an algorithm-type artificial neural network, that will be utilized to classify gene-expression changes associated with neuroinflammatory conditions. P14 has already collected comprehensive gene expression data from four different immune-mediated and neurodegenerative pathologies affecting the CNS in the C57BL/6 mouse strain. Specifically, four well-established mouse models representing the major diseases that affect the human CNS in developed countries were chosen, namely Alzheimer’s disease (represented by transgenic mice overexpressing human amyloid precursor protein), multiple sclerosis (experimental autoimmune encephalomyelitis/EAE and Tg6074 TNF transgenic mice) and cerebral stroke (permanent middle cerebral artery occlusion). Strict standardization of RNA/cDNA samples and data collection was followed to minimize variables such as genetic background (C57BL/6 chosen), tissue composition (left-half-brain samples), disease stage (time points covering initiation, development and resolution of disease) and control choice (normal littermate versus single reference cDNA) so as to follow MIAME criteria [minimal information about a microarray experiment, Brazma et al., Nature Genetics (2001) 29(4): 365-371] and to allow data submission to public data bases such as the EBI Database “ArrayExpress”. Gene expression data was obtained using a 27K cDNA microarray representing a
significant fraction of the genes encoded within the mouse genome, in the framework of a collaboration with Dr John Quackenbush, TIGR, USA. During the first 18 months of the project, the P14 team aims to utilize the high-throughput, time-course expression data generated from the expression profiling of each disease separately to construct an algorithm that will be built to robustly classify gene-expression changes into the ones that commonly characterize neuroinflammatory conditions and others that lead to and characterize specific brain pathologies. Although the method of choice will be artificial neural networks, a number of alternative approaches, including simple linear discriminant analysis, k-nearest neighbors, and a variety of weighted voting schemes will also be investigated. Data will be normalized, filtered and analyzed to identify subsets of genes that best distinguish each specific phenotype from the others. The union of all genes identified in this way will be used as an initial classification set for algorithm development. The first 18 months of the project will be devoted to develop and train the algorithm to differentiate between the disease-specific (diagnostic) components of each of the four selected mouse disease models. For initial validation, the dataset will be divided into randomly selected training and test sets (75% and 25%, respectively), and these will be used to build and validate a number of independent classifiers. Should the classifier prove accurate, randomly selected murine brain lesions from the target neuroinflammatory conditions will be profiled on arrays and used to test the performance of the built algorithm. Following this initial phase, the network/algorithm will be further educated with data sets from new mouse disease models utilized by the consortium participants, with the aim of increasing its sensitivity and of obtaining a working platform that will allow to differentiate between related and unrelated neuroinflammatory diseases and to evaluate the efficacy of experimental therapeutic regimens. These activities will extend beyond 18 months.

Gantt charts showing the timing of the different WPs and their tasks

The next five pages depict the Gantt charts of the four scientific subprojects.

- Subproject **Identification**: Workpackages I1 to I6
- Subproject **Mechanisms**: Workpackages M1 to M4
- Subproject **Validation**: Workpackages V1 to V7
- Subproject **Horizontal Integration**: Workpackages H1 to H2
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<th>WP-V</th>
<th>Genomics of inflammatory neurodegeneration in DAP12 and TREM-2 mutant mice</th>
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<th>&gt; 18</th>
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<tr>
<td>DE-V.1</td>
<td>Gene expression profiles of DAP12 and TREM-2 mutant mice at distinct disease stages</td>
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<td>DE-V.2</td>
<td>Fully analyzed data set listing key genes and/or pathways associated with disease progression in DAP12 and TREM-2 mutant mice</td>
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<td>WP-M1</td>
<td>CD8+ T-cell-mediated neurotoxicity in humanized animal models</td>
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<td>DE-M1.1</td>
<td>Development of standardised animal models for the analysis of CD8+ T cell-mediated CNS tissue damage</td>
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<td>DE-M1.2</td>
<td>Transgenic mice in which the expression level of the target CNS autoantigen is controlled by administration of doxycycline</td>
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<td>DE-M1.3</td>
<td>Identification of molecules and pathways involved in neuronal/axonal or glial cell damage</td>
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<tr>
<th>WP-M2</th>
<th>Epistatic interactions in the MS-associated DR2 haplotype in humanized mouse models</th>
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<tr>
<td>DE-M2.1</td>
<td>Triple transgenic mice expressing human MHC class II molecules (DR15 and/or DR51) and three different human T cell receptors solely or dually restricted by these MHC molecules</td>
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<tr>
<td>DE-M2.2</td>
<td>Soluble MS-associated MHC class II molecules</td>
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<tr>
<td>DE-M2.3</td>
<td>Soluble MS-associated T cell receptors</td>
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<td>DE-M2.4</td>
<td>DR15-MBP tetramers</td>
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<th>WP-M3</th>
<th>Intracerebral lymphoid tissue in CNS tissue injury</th>
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<tr>
<td>DE-M3.1</td>
<td>Identification, characterization and collection of CNS germinal centers from MS brain tissue</td>
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<td>DE-M3.2</td>
<td>Lentiviral vectors expressing BAFF and CXCL13</td>
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<td>DE-M3.3</td>
<td>Animal models for the analysis of CNS compartmentalized immune responses</td>
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<th>WP-M6</th>
<th>Proteomics of axonal degeneration in a model of optic nerve transection</th>
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<tr>
<td>DE-M6.1</td>
<td>Reproducible model of experimental Wallerian degeneration</td>
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<td>DE-M6.2</td>
<td>Proteomic analysis of degenerating fibre tract</td>
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<tr>
<td>DE-M6.3</td>
<td>Influence of environment on proteomic profile of degenerating axons</td>
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Annex-1 – “Description of work”, Page 62 of 155
<table>
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<tr>
<th>WP-M4</th>
<th>Mechanisms and key intracellular regulators of microglia-mediated inflammatory axonal injury</th>
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<td>Knowledge on the involvement of microglial inflammatory mediators in axonal transport disturbance and axonal injury</td>
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<td>DE-M4.2</td>
<td>Demonstration of the signaling pathways induced in axons by microglial inflammatory mediators</td>
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<td>WP-V1</td>
<td>New therapeutics targeting the TREM-2 pathway</td>
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<tr>
<td>DE-V1.1</td>
<td>Report on the effects of anti-TREM-2 monoclonal antibodies in EAE</td>
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<td>DE-V1.2</td>
<td>Report on the effects of TREM-2/IgG fusion proteins in EAE</td>
<td>13</td>
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<td>DE-V1.3</td>
<td>Report on TREM-2 stimulation and effects in cultured microglia</td>
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<td>WP-V2</td>
<td>New therapeutics targeting the Ncf1 pathway</td>
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<tr>
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<td>New genetic targets identified useful for therapy from the analysis of the Ncf1 pathway</td>
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<td>DE-V2.2</td>
<td>New patented compounds with oxidant inducing activity in vivo displaying neuroprotective effects in animal models</td>
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<td>DE-V2.3</td>
<td>Genetic biomarkers for anti-inflammatory effects of Ncf1 targeting new drugs</td>
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<td>WP-V3</td>
<td>New therapeutics selective for TNF receptor subtypes and humanized animal models</td>
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<td>DE-V3.1</td>
<td>TNFR2 selective agonists</td>
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<td>DE-V3.2</td>
<td>TNFR1 receptor antagonists</td>
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<td>DE-V3.3</td>
<td>Recombination positive ES clones for human TNFR1 and TNFR2</td>
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<td>WP-V4</td>
<td>Small drug molecules targeting the neuronal TNFR1 core signaling scaffold</td>
<td>Start month</td>
<td>End month</td>
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<tr>
<td>DE-V4.1</td>
<td>Detailed knowledge of neuronal TNFR1-mediated neuroprotective signalling</td>
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<td>DE-V4.2</td>
<td>Identification of cross-talk with neurodegenerative pathways (e.g., JNK-mediated, glutamate) and determination of the mechanism of neuroprotection</td>
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<td>DE-V4.3</td>
<td>Development and screening of cell-penetrating peptides designed to have neuroprotective function</td>
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<td>DE-V4.4</td>
<td>Combination therapies of peptides with TNFR agonists and antagonists and other relevant neuroprotective compounds</td>
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<td>WP-V5</td>
<td>Axonal protection by sodium channel and sodium exchanger blocking compounds</td>
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<tr>
<td>DE-V5.1</td>
<td>Sodium channel blockers that are most effective in protecting axons from degeneration in in vitro and semi in vivo models</td>
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<td>Drugs that inhibit the reverse mode of operation of the sodium-calcium exchanger and are most effective in protecting axons from degeneration in in vitro and semi in vivo models</td>
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<td>WP-V6</td>
<td>Axonal protection by glutamate receptor and calcium channel blocking compounds</td>
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<td>DE-V6.1</td>
<td>Establishment of various EAE models to be utilised for in vivo screening (months 1-6)</td>
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<td>DE-V6.2</td>
<td>Establishment of calcium imaging methods in ex-vivo spinal cord slice</td>
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<td>Definition of parameters of glutamatergic channel signalling in axonal degeneration in EAE</td>
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<td>WP-V7</td>
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<td>DE-V7.1</td>
<td>EAE models with strong neurodegenerative aspects for therapeutic treatment evaluation</td>
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<td>DE-V7.2</td>
<td>In vivo electrophysiology in rodents</td>
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<td>Fusion proteins for transduction of anti-apoptotic proteins</td>
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<td>WP-H1 Neuropathology Reference Center</td>
<td>Start month</td>
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<td>DE-H1.1 Quantitative characterization of human brain lesions in biopsy and autopsy material in relation to inflammation, demyelination, remyelination and axonal injury</td>
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<td>DE-H1.2 DNA isolation from archival biopsy and autopsy tissue</td>
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<td>DE-H1.3 Expression studies of newly identified targets in human tissue in relation to specific aspects of pathology</td>
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<td>DE-H1.4 Neuropathological analysis of experimental models</td>
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<td>DE-H2.3 Fully validated computer-based algorithm representing gene changes associated with 4 independent mouse models</td>
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8.3 GRAPHICAL PRESENTATION OF WORKPACKAGES

RTD/INNOVATION ACTIVITIES

Subproject Identification

I1 Mouse genetic platform for the identification of neuroinflammatory disease-related genes
I2 Rat genetic platform for the identification of neuroinflammatory disease-related genes
I3 Identification of susceptibility genes in MS patients
I4 Genomics of axonal degeneration in myelin mutant mice
I5 Genomics of inflammatory neurodegeneration in DAP12 and TREM-2 mutant mice
I6 Proteomics of axonal degeneration in a model of optic nerve transection

Subproject Mechanisms

M1 CD8+ T-cell-mediated neurotoxicity in humanized animal models
M2 Epistatic interactions in the MS-associated DR2 haplotype
in humanized mouse models
M3 Intracerebral lymphoid tissue in CNS tissue injury
M4 Mechanisms and key intracellular regulators of microglia-mediated inflammatory axonal injury

Subproject Validation

V1 New therapeutics targeting the TREM-2 pathway
V2 New therapeutics targeting the Ncf1 pathway
V3 New therapeutics selective for TNF receptor subtypes and humanized animal models
V4 Small drug molecules targeting the neuronal TNFR1 core signaling scaffold
V5 Axonal protection by sodium channel and sodium exchanger blocking compounds
V6 Axonal protection by glutamate receptor and calcium channel blocking compounds
V7 Combination therapies with neuroprotective anti-apoptotic and anti-inflammatory drugs

Subproject Training

T1 Educational training programme
T2 Training workshops

Subproject Management

Scientific, administrative and business management
8.4 WORKPACKAGE LIST

Detailed work description broken down into workpackages

- Subproject **Identification**: Workpackages I1 to I6
- Subproject **Mechanisms**: Workpackages M1 to M4
- Subproject **Validation**: Workpackages V1 to V7
- Subproject **Horizontal Integration**: Workpackages H1 to H2
- Subproject **Training**: Workpackages T1 to T2
- Subproject **Management**: Workpackage MAN

### Workpackage list (18 month plan)

<table>
<thead>
<tr>
<th>Workpackage No.</th>
<th>Workpackage title</th>
<th>Lead contractor No.</th>
<th>Person-months</th>
<th>Start month</th>
<th>End month</th>
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<tr>
<td>I1</td>
<td>Mouse genetic platform for the identification of neuroinflammatory disease-related genes</td>
<td>P3</td>
<td>72</td>
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<td>I3</td>
<td>Identification of susceptibility genes in MS patients</td>
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<td>I4</td>
<td>Genomics of axonal degeneration in myelin mutant mice</td>
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<td>Proteomics of axonal degeneration in a model of optic nerve transection</td>
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1 Workpackage number: WP 1 – WP n.
2 Number of the contractor leading the work in this workpackage.
3 The total number of person-months allocated to each workpackage.
4 Relative start date for the work in the specific workpackages, month 0 marking the start of the project, and all other start dates being relative to this start date.
5 Relative end date, month 0 marking the start of the project, and all ends dates being relative to this start date.
6 Deliverable number: Number for the deliverable(s)/result(s) mentioned in the workpackage: D1 - Dn.
| M1 | CD8+-T-cell-mediated neurotoxicity in humanized animal models | P6 | 62 | 1 | > 18 | DE-M1.1, DE-M1.2, DE-M1.3 |
| M2 | Epistatic interactions in the MS-associated DR2 haplotype in humanized mouse models. | P4 | 52 | 1 | > 18 | DE-M2.1, DE-M2.2, DE-M2.3, DE-M2.4 |
| M3 | Intracerebral lymphoid tissue in CNS tissue injury. | P1 | 72 | 1 | > 18 | DE-M3.1, DE-M3.2, DE-M3.3 |
| M4 | Mechanisms and key intracellular regulators of microglia-mediated inflammatory axonal injury. | P19 | 50 | 1 | > 18 | DE-M4.1, DE-M4.2 |
| V1 | New therapeutics targeting the TREM-2 pathway | P19 | 24 | 7 | > 18 | DE-V1.1, DE-V1.2, DE-V1.3 |
| V2 | New therapeutics targeting the Ncf1 pathway. | P17 | 90 | 1 | > 18 | DE-V2.1, DE-V2.2, DE-V2.3 |
| V3 | New therapeutics selective for TNF receptor subtypes and humanized animal models | P12 | 123 | 1 | > 18 | DE-V3.1, DE-V3.2, DE-V3.3 |
| V4 | Small drug molecules targeting the neuronal TNFR1 core signaling scaffold | P14 | 46 | 1 | > 18 | DE-V4.1, DE-V4.2, DE-V4.3, DE-V4.4 |
| V5 | Axonal protection by sodium channel and sodium exchanger blocking compounds | P11 | 45 | 1 | > 18 | DE-V5.1, DE-V5.2 |
| V6 | Axonal protection by glutamate receptor and calcium channel blocking compounds | P18 | 45 | 1 | > 18 | DE-V6.1, DE-V6.2, DE-V6.3 |
| V7 | Combination therapies with neuroprotective anti-apoptotic and anti-inflammatory drugs | P2a | 60 | 1 | > 18 | DE-V7.1, DE-V7.2, DE-V7.3 |
| H1 | Neuropathology Reference Center | P2b, P5 | 80 | 1 | >18 | DE-H1.1, DE-H1.2, DE-H1.3, DE-H1.4 |
| H2          | Genomics/Proteomics Reference Centers | P3, P10 | 44 | 1 | >18 | DE-H2.1  
|            |                                      |         |    |   |     | DE-H2.2  
|            |                                      |         |    |   |     | DE-H2.3  
| T1         | Educational Training Programme       | P14     | 1,5| 7 | >18 | DE-T1.1  
|            |                                      |         |    |   |     | DE-T1.2  
|            |                                      |         |    |   |     | DE-T1.3  
|            |                                      |         |    |   |     | DE-T1.4  
| T2         | Training Workshops                   | P2a     | 1,5| 7 | >18 | DE-T2.1  
|            |                                      |         |    |   |     | DE-T1.2  
|            |                                      |         |    |   |     | DE-T1.3  
| MAN        | Management                           | P1      | 36 | 1 | >18 | DE-MAN.1 
|            |                                      |         |    |   |     | DE-MAN.2 
|            |                                      |         |    |   |     | DE-MAN.3 
|            |                                      |         |    |   |     | DE-MAN.4 
|            |                                      |         |    |   |     | DE-MAN.5 
|            |                                      |         |    |   |     | DE-MAN.6 
|            |                                      |         |    |   |     | DE-MAN.7 
|            |                                      |         |    |   |     | DE-MAN.8 
|            |                                      |         |    |   |     | DE-MAN.9 
|            |                                      |         |    |   |     | DE-MAN10 
|            |                                      |         |    |   |     | DE-MAN11 
|            |                                      |         |    |   |     | DE-MAN12 
|            |                                      |         |    |   |     | DE-MAN13 
| TOTAL      |                                     |         |    |   |     | 1253     |
8.5 DELIVERABLES LIST (18 MONTH PLAN)

<table>
<thead>
<tr>
<th>Del. No.</th>
<th>Deliverable name</th>
<th>WP no.</th>
<th>Lead participant</th>
<th>Estimated indicative persons months</th>
<th>Nature</th>
<th>Dissemination level</th>
<th>Delivery date (Proj. month)</th>
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<tr>
<td>DE-I1.1</td>
<td>Report on minimized congenic mouse strains containing less than 10 genes that control essential pathways of EAE</td>
<td>I1</td>
<td>P3</td>
<td>20</td>
<td>R</td>
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<td>DE-I1.2</td>
<td>Report on a partial advanced intercross line with defined interchromosomal and extrachromosomal interaction between defined candidate genes</td>
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<td>P3</td>
<td>14</td>
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<td>12 mo</td>
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<td>DE-I1.3</td>
<td>Report on a new positionally cloned gene harbouring polymorphic effects on development of EAE</td>
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<td>P3</td>
<td>20</td>
<td>R</td>
<td>CO</td>
<td>18 mo</td>
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<td>DE-I1.4</td>
<td>Attempt to validate at least one of these genes confirming them as appropriate drug targets and thereby defending patent applications</td>
<td>I1</td>
<td>P3</td>
<td>18</td>
<td>R</td>
<td>CO</td>
<td>18 mo</td>
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<tr>
<td>DE-I2.1</td>
<td>Report on the establishment of at least 5 minimized congenic rat strains, containing less than 10 genes, and their genetic interactive behaviour</td>
<td>I2</td>
<td>P8</td>
<td>30</td>
<td>R</td>
<td>CO</td>
<td>18 mo</td>
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</table>

7 Deliverable numbers in order of delivery dates: D1 – Dn
8 Please indicate the nature of the deliverable using one of the following codes:
   R = Report
   P = Prototype
   D = Demonstrator
   O = Other
9 Please indicate the dissemination level using one of the following codes:
   PU = Public
   PP = Restricted to other programme participants (including the Commission Services)
   RE = Restricted to a group specified by the consortium (including the Commission Services)
   CO = Confidential, only for members of the consortium (including the Commission Services)
10 Month in which the deliverables will be available. Month 0 marking the start of the project, and all delivery dates being relative to this start date.
<p>| DE-12.2 | Report on a genome wide genetic interaction study based on the AIL cross for the functional expression of Ncf1 polymorphism | I2 | P8 | 30 | R | CO | 18 mo |
| DE-12.3 | Understanding how evolutionary conserved polymorphisms in CIITA affect neurodegenerative disease, including rat strains congenic for this genome region, available as pharmaco-genomically defined strains | I2 | P8 | 24 | R | CO | 18 mo |
| DE-12.4 | Positional cloning of at least 2 new genes responsible for major QTL effects affecting EAE or neurodegeneration | I2 | P8 | 42 | R | CO | 18 mo |
| DE-13.1 | Establishment of a database structure and dataset of more than 4,000 individuals (2,000 patients, 2,500 family members and controls) containing phenotypic information | I3 | P8/P9 | 45 | O | CO | 9 mo |
| DE-13.2 | Full genotypic characterization of known MS risk genes in the above data-set (HLA class II, class I, PRKCA, CIITA and CTLA-4) | I3 | P8/P9 | 45 | R | CO | 9 mo |
| DE-13.3 | Report on gene-gene interaction analysis of the above mentioned risk genes in the data-set | I3 | P8/P9 | 48 | R | CO | 18 mo |
| DE-14.1 | Gene expression profiles (raw data) of neuronal and glial cell populations at different stages of axonal pathology | I4 | P7 | 28 | R | CO | 18 mo |
| DE-14.2 | Fully analyzed data set listing key genes and/or pathways contributing to initial and late stages of axonal degeneration in ‘myelin’ mutant mice | I4 | P7 | 6 | R | CO | 18 mo |
| DE-I5.1 | Gene expression profiles of DAP12 and TREM-2 mutant mice at distinct disease stages | I5 | P19 | 24 | R | CO | 18 mo |
| DE-I5.2 | Fully analyzed data set listing key genes and/or pathways associated with disease progression in DAP12 and TREM-2 mutant mice | I5 | P19 | 6 | R | CO | 18 mo |
| DE-I6.1 | Reproducible model of experimental Wallerian degeneration | I6 | P10 | 8 | R | CO | 6 mo |
| DE-I6.2 | Proteomic analysis of degenerating fibre tract | I6 | P10 | 6 | R | CO | 12 mo |
| DE-I6.3 | Influence of environment on proteomic profile of degenerating axons | I6 | P10 | 7 | R | CO | ≥ 18 mo |
| DE-M1.1 | Development of standardised animal models for the analysis of CD8+ T cell-mediated CNS tissue damage | M1 | P6 | 26 | O | CO | 12 mo |
| DE-M1.2 | Transgenic mice in which the expression level of the target CNS autoantigen is controlled by administration of doxycycline | M1 | P6 | 18 | O | CO | 18 mo |
| DE-M1.3 | Identification of molecules and pathways involved in neuronal/axonal or glial cell damage | M1 | P6 | 18 | R | CO | ≥ 18 mo |
| DE-M2.1 | Triple transgenic mice expressing human MHC class II molecules (DR15 and/or DR51) and three different human T cell receptors solely or dually restricted by these MHC molecules | M2 | P4 | 22 | O | CO | 18 mo |
| DE-M2.2 | Soluble MS-associated MHC class II molecules | M2 | P4 | 12 | P | CO | 18 mo |
| DE-M2.3 | Soluble MS-associated T cell receptors | M2 | P4 | 12 | P | CO | 18 mo |
| DE-M2.4 | DR15-MBP tetramers | M2 | P4 | 6 | P | CO | 12 mo |</p>
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<th>36</th>
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<th>18 mo</th>
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<td>Identification, characterization and collection of CNS germinal centers from MS brain tissue</td>
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<td>Lentiviral vectors expressing BAFF and CXCL13</td>
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<td>P1</td>
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<td>Animal models for the analysis of CNS compartmentalized immune responses</td>
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<td>Knowledge on the involvement of microglial inflammatory mediators in axonal transport disturbance and axonal injury</td>
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<td>Report on the effects of anti-TREM-2 monoclonal antibodies in EAE</td>
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<td>Report on the effects of TREM-2/IgG fusion proteins in EAE</td>
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<td>6</td>
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<td>DE-V1.3</td>
<td>Report on TREM-2 stimulation and effects in cultured microglia</td>
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<td>DE-V2.2</td>
<td>New patented compounds with oxidant inducing activity in vivo displaying neuroprotective effects in animal models</td>
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<td>DE-V2.3</td>
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<td>DE-V3.1</td>
<td>TNFR2 selective agonists</td>
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<td>Identification of cross-talk with neurodegenerative pathways (e.g. JNK-mediated, glutamate) and determination of the mechanism of neuroprotection</td>
<td>V4</td>
<td>P14</td>
<td>14</td>
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<td>CO</td>
<td>12 mo</td>
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<tr>
<td>DE-V4.3</td>
<td>Development and screening of cell-penetrating peptides designed to have neuroprotective function</td>
<td>V4</td>
<td>P14</td>
<td>12</td>
<td>P/R</td>
<td>CO</td>
<td>18 mo</td>
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<td>DE-V4.4</td>
<td>Combination therapies of peptides with TNFR agonists and antagonists and other relevant neuroprotective compounds</td>
<td>V4</td>
<td>P14</td>
<td>6</td>
<td>R</td>
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<td>&gt; 18 mo</td>
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<td>DE-V5.1</td>
<td>Sodium channel blockers that are most effective in protecting axons from degeneration in \textit{in vitro} and semi \textit{in vivo} models</td>
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<td>P11</td>
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<td>CO</td>
<td>18 mo</td>
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<tr>
<td>DE-V5.2</td>
<td>Drugs that inhibit the reverse mode of operation of the sodium-calcium exchanger and are most effective in protecting axons from degeneration in \textit{in vitro} and semi \textit{in vivo} models</td>
<td>V5</td>
<td>P11</td>
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<td>CO</td>
<td>18 mo</td>
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<tr>
<td>DE-V6.1</td>
<td>Establishment of various EAE models to be utilised for \textit{in vivo} screening</td>
<td>V6</td>
<td>P18</td>
<td>15</td>
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<td>CO</td>
<td>6 mo</td>
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<tr>
<td>DE-V6.2</td>
<td>Establishment of calcium imaging methods in ex-\textit{vivo} spinal cord slice</td>
<td>V6</td>
<td>P18</td>
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<td>R</td>
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<td>DE-V6.3</td>
<td>Definition of parameters of glutamatergic/ion channel signalling in axonal degeneration in EAE</td>
<td>V6</td>
<td>P18</td>
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<td>18 mo</td>
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<td>DE-V7.1</td>
<td>EAE models with strong neurodegenerative aspects for therapeutic treatment evaluation</td>
<td>V7</td>
<td>P2a</td>
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<td>DE-V7.2</td>
<td>In vivo electrophysiology in rodents</td>
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<td>DE-V7.3</td>
<td>Fusion proteins for transduction of anti-apoptotic proteins</td>
<td>V7</td>
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<td>6 mo</td>
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<td>DE-H1.1</td>
<td>Quantitative characterization of human brain lesions in biopsy and autopsy material in relation to inflammation, demyelination, remyelination and axonal injury</td>
<td>H1</td>
<td>P2b/P5</td>
<td>30</td>
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<td>CO</td>
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<tr>
<td>DE-H1.2</td>
<td>DNA isolation from archival biopsy and autopsy tissue</td>
<td>H1</td>
<td>P2b</td>
<td>12</td>
<td>R</td>
<td>CO</td>
<td>18 mo</td>
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<tr>
<td>DE-H1.3</td>
<td>Expression studies of newly identified targets in human tissue in relation to specific aspects of pathology</td>
<td>H1</td>
<td>P2b/P5</td>
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<td>CO</td>
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<td>DE-H1.4</td>
<td>Neuropathological analysis of experimental models</td>
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<tr>
<td>DE-H2.1</td>
<td>Gene expression profiles of animal models of disease</td>
<td>H2</td>
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<td>DE-H2.2</td>
<td>Protein expression profiles of animal models of disease</td>
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<td>T1</td>
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<td>DE-T1.2</td>
<td>Calls for candidates for Marie Curie Fellowships</td>
<td>T1</td>
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<td>DE-T2.1</td>
<td>Programme of thematic workshops and technical meetings (month 12)</td>
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<td>First Steering Committee meeting</td>
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<td>P1/P19</td>
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<td>O</td>
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<td>6 mo</td>
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<td>DE-MAN.5</td>
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<td>MAN</td>
<td>P1/P19</td>
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<td>Submission of the next 18 month Detailed Implementation Plan to the EC</td>
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<td>DE-MAN.10</td>
<td>Presentation of abstracts to national and international meetings and submission of publications to peer-reviewed journals</td>
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8.6 WORKPACKAGE DESCRIPTION

Subproject Identification (Workpackage I1)

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Objectives
To identify new disease-related genes using a mouse EAE genetic platform

Description of work
We have since several years built an animal model platform suitable for identifying the relevant genes for inflammatory autoimmune diseases. It is based on the identification of the major loci controlling development of well-defined models of MS and RA. All these loci have been isolated in so called congenic strains, which requires years of selective breeding but which are extremely useful for the next step in the analysis. All the congenic fragments have been placed in the C57/Black genetic background for which genomic sequence and ES cells are available. This is also a major advantage as it allows comparison of the data and direct studies on interactions. In addition, we have selected and made several new transgenics, mutations, knockouts and knockins, that are all bred into the same genetic background. This gives us an advantage in the analysis of the pathways caused by the identified genes. Taken together, the animal platform is internationally unique and consists of 12000 mice and over 150 unique strains.

The strategy to isolate the genes will be based on selective recombinational selection in congenic strains using a strategy we already have successfully demonstrated that we can positionally clone genes in QTL (so far we have published the Ncf1 and the Aq genes). We have already mapped the major loci containing genes controlling chronic relapsing EAE, mainly based on crosses between RIIS/J and B10.RIII. These loci have been isolated in well defined congenic strains. We now have 15 different strains and we will now aim to positionally clone the genes in these loci through 1) minimising the congenic fragment in selected congenics; 2) developing and analysing genetic interactions in partial advanced intercrosses; 3) analysing expression pattern of all genes within the selected congenics; 4) identifying the responsible polymorphism by sequence analysis; 5) reproducing the genetic polymorphism using ES cell and lentivirus based technology; and 6) analysing the functional pathway controlled by the cloned genes and transferring these results to collaborators within the consortium to study their role in MS and for target validation together with selected European SMEs.

Deliverables
DE-I1.1 Report on minimized congenic mouse strains containing less than 10 genes that control essential pathways of EAE (18 mo).

DE-I1.2 Report on a finalized partial advanced intercross line with defined interchromosomal and extrachromosomal interaction between defined candidate genes (12 mo)

DE-I1.3 Report of a new positionally cloned gene harbouring polymorphic effects on development of EAE (18 mo)

DE-I1.4 Attempt to validate at least one of these genes confirming them as appropriate drug targets and thereby defending patent applications (18 mo)
### Milestones and expected result

<table>
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<th>Milestone</th>
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<tr>
<td>MI-I1.1</td>
<td>Selection of 5 congenic mouse strains suitable for positional cloning of the underlying polymorphic genes</td>
</tr>
<tr>
<td>MI-I1.2</td>
<td>Identification of new genes and pathways of importance for EAE</td>
</tr>
<tr>
<td>MI-I1.3</td>
<td>Establishment of new models for MS based on the genetic discoveries.</td>
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11 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Identification (Workpackage I2)

<table>
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- P3
- P5
- P8

**Person-months per participant**
- 36 (See H1)
- 90

**Objectives**
To identify new EAE- and neurodegeneration-related genes using a rat genetic platform.

**Description of work:**
Using well defined models for EAE in rats we have isolated the most important loci in congenic strains, mainly from crosses using the DA rat strain as the susceptible partner. These congenic strains will now be used to identify the underlying genes and to explore the pathways they control. The development of EAE involves several essential pathways involving interactions between the immune system and the CNS and lastly a chronic neurodegeneration. As MS, the animal model EAE is a complex disease and for isolating the genes we will mainly use a congenic strategy combined with well established advanced intercross lines. For studies of neurodegeneration used substrats, we will also use a model with local neurodegeneration and activation of CNS innate immunity, induced by ventral nerve root avulsion (VRA) (Lidman et al., J. Neurosci. 2003; 23: 9817). These are now fine-mapped in an advanced intercross line (AIL) between the DA and PVG strains. Additional recombinational selection of congenic strains, gene sequencing, haplotype analysis and expression analysis can allow exact gene positioning with definition of disease-driven genes attractive as drug targets.

Using this strategy, we have recently identified a functional polymorphism in the CIITA gene controlling MHC class II expression. After comparative genomics, a SNP in promotor III of CIITA has shown in association to MS, as well as two other diseases with inflammatory components (Swanberg et al, pending revision Nature Genetics). We now have the tools in form of reciprocal congenics for the CIITA region in the rat, which allow mechanistic studies in a variety of EAE and neurodegeneration models. These studies will permit mechanistic understanding of how this polymorphism regulates disease.

For other loci regulating neuronal vulnerability and T-cell accumulation in the CNS, gene positioning studies are ongoing.

**Deliverables:**
- DE-I2.1 Report on the establishment of at least 5 minimized congenic rat strains, containing less than 10 genes controlling EAE, and their genetic interactive behavior (18 mo)
- DE-I2.2 Report on a genome wide genetic interaction study based on the AIL cross for the functional expression of Ncf1 polymorphism (18 mo)
- DE-I2.3 Understanding how evolutionary conserved polymorphisms in CIITA affect neurodegenerative disease, including rat strains congenic for this genome region, available as pharmaco-genomically defined strains (18 mo)
- DE-I2.4 Positional cloning of at least 2 new genes responsible for major QTL effects affecting EAE or neurodegeneration (18 mo)
Milestones\textsuperscript{12} and expected result

MI-I2.1 Selection of 5 congenic rat strains suitable for positional cloning of the underlying polymorphic genes

MI-I2.2 Identification of new genes and pathways of importance for EAE suitable for a validated target approach for development of new drugs

MI-I2.3 Establishment of new models for MS based on the genetic discoveries

\textsuperscript{12} Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Identification (Workpackage I3)

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Objectives
Identification of susceptibility genes and gene-gene interaction in MS

Description of work
The genetic analyses to be performed in this workpackage will take advantage of the special character of two unique MS populations, the genetically original and relatively isolated Finnish MS population including many MS families, and the high prevalence Stockholm MS population, representing the supposed Scandinavian founder population for MS in Europeans (“the Viking hypothesis”).

We propose to establish a large dataset of 1,500 unrelated Swedish MS patients and ethnically matched controls and over 700 Finnish MS families, each individual clinically characterised and genotyped for known risk genes. A gene-gene interaction analysis will reveal novel information and allow assessment of further genes which will be identified in other WPs within the Subproject Identification. High-throughput SNP genotyping with densely spaced markers (1 per 1-2 kilo base pairs) will be performed for selected genes.

Data management is key to this project together with development of statistical methods. Clinical information is available from regional and national MS registries. Background medical information, data regarding socioeconomic and health-related factors can be obtained from public Swedish and Finnish national registries allowing assessment of gene-environment interaction, thus increasing the potential of this project from a societal perspective.

Deliverables
DE-I3.1 Establishment of a database structure and dataset of more than 4,000 individuals (2,000 patients, 2,500 family members and controls) containing phenotypic information (9 mo)
DE-I3.2 Full genotypic characterization of known MS risk genes in the above data-set (HLA class I and class II, PRKCA, CIITA and CTLA-4) (9 mo)
DE-I3.3 Report on the gene-gene interaction analysis of the above mentioned risk genes in the data-set (18 mo)

Milestones and expected result
MI-I3.1 Completion of physical data-set and data-base structure
MI-I3.2 Completion of genotyping for known risk genes
MI-I3.3 Completion of gene-gene-interaction analysis

The power of the data-set, and the importance of previously identified risk genes in the full data-set will influence the gene-gene interaction analysis and set the basis for the evaluation of new candidate risk genes. Milestone I3.2 will create a unique platform enabling successful identification of new genes.

13 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Identification (Workpackage I4)

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Objectives
To identify the molecular pathways and monitor altered gene expression that is associated with axonal damage in myelin mutant mice. In particular, we shall define the gene expression profiling of defined projection neurons and oligodendrocytes in dysmyelinated genetic mouse models.

Description of work
Well characterized mice deficient in myelin-specific genes (CNP, PLP) will be used to identify the molecular pathways and monitor altered gene expression that is associated and possibly critical for the axonal damage. These mice undergo severe neurodegenerative changes, including axonal swellings and loss, but in the presence of nearly normal CNS myelin. The onset and severity of the axonal phenotypes is highly reproducible and allows to identify the neuronal changes that precede axonal loss.

We have developed a technical platform that enables us, following a transgenic labelling of cell nuclei, to isolate defined projection neurons from the cortex and cerebellum of mouse brain sections by fluorescence-directed laser microdissection. With this strategy, we can perform Affymetrix gene chip analysis from defined cell types with high reproducibility, using a few as 50 individual cells. This technique provides the prerequisite to perform global transcriptome analysis in a maximally unbiased approach, focusing on neurons and oligodendrocytes isolated from mutant mice with progressive neurodegeneration. The availability of different ‘myelin’ mutant mice with similar axonal and neurological phenotypes places us in a position to cross-control our microarray results, and to cross-validate potential therapeutic interventions in the future.

Deliverables
DE-I4.1: Gene expression profiles (raw data) of: (I) large projection neurons from layer V of the motor-cortex, (II) oligodendrocytes obtained from the corpus callosum, (III) Purkinje cells from the cerebellum and (IV) oligodendrocytes from the underlying cerebellar white matter at different stages of axonal pathology (18 mo)
DE-I4.2: Fully analyzed data set listing key genes and/or pathways contributing to initial and late stages of axonal degeneration in ‘myelin’ mutant mice (18 mo)

Milestones and expected result
MI-I4.1 Unbiased gene expression profiles of ‘affected’ neurons and ‘causing’ oligodendrocytes from myelin mutant and control mice
MI-I4.2 Identification of candidate genes or molecular pathways involved in axonal degeneration.

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14 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
**Subproject Identification (Workpackage I5)**

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**Objectives**
To identify genes and molecular pathways associated with neurodegeneration in DAP12 and TREM-2 mutant mice.

**Description of work**
TREM2 is a member of the innate immune receptor TREM-family and signals via the ITAM-containing adaptor protein DAP12. Genetic mutations (DAP12 and TREM-2) have been recently described by P9 (NPHI) in patients with PLOSL/Nasu-Hakola demonstrating that dysfunction of TREM-2/DAP12, expressed in microglial cells, results in synaptic impairment and chronic inflammatory neurodegeneration. TREM-2 knockout and shRNA interference TREM-2 knockdown mice were established and are available for analysis in the laboratories of P9 and P19. The DAP12 mutant mice have already been carefully analyzed showing a very late onset and mild CNS phenotype with thalamic hypomyelinoses, synaptic dysfunction and synapse loss. However, the exact molecular mechanism and the genes/molecules involved in the CNS impairment remain unknown.

Gene expression profiling will be performed in DAP12 and TREM-2 mutant mice at early and late disease stages to identify new genes/molecules involved in inflammatory neurodegeneration (late disease stage) and synaptic dysfunction (early disease stage).

RNA will be isolated from distinct brain regions at defined age of mutant and control mice. Labeled RNA will be hybridized to Affymetrix whole genome microarrays. Validation of genes involved in inflammatory neurodegeneration will be performed by quantitative real-time PCR and *in situ* hybridization in the mutant mice at distinct time points.

**Deliverables**

- DE-I5.1 Gene expression profiles of DAP12 and TREM-2 mutant mice at distinct disease stages (18 mo)
- DE-I5.2: Fully analyzed data set listing key genes and/or pathways associated with disease progression in DAP12 and TREM-2 mutant mice (18 mo)

**Milestones**

- MI-I5.1 Gene expression profiles of DAP12/TREM-2 mutant and control mice
- MI-I5.2 Identification of molecular targets for therapeutic intervention of inflammatory neurodegeneration

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15 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Identification (Workpackage I6)

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Objectives
To analyze the changes in global protein expression profile that underpin the early stages of axon degeneration in a model of optic nerve transection.

Description of work
We will use a mouse model of axon degeneration, transection of the optic nerve, to induce Wallerian degeneration in a defined axon population with well defined time course of degeneration of different populations of axons and a well defined glial response. We will use a proteomic approach in collaboration with the Centre for Proteomics, University of Southampton, to identify changes in proteins during axon degeneration. We will use Isotope Coded Affinity Tag (ICAT) to identify the protein profile. ICAT has the advantage that it provides a quantitative estimate of the changes in particular proteins and is also a powerful method for the detection of post-translational modifications.

We will also investigate how the proteomic profile, including proteins from degenerating axons and components of glial cells, is influenced by a systemic inflammatory challenge, a stimulus known to induce a significant number of relapses in patients with MS.

Deliverables
DE-I6.1: Reproducible model of experimental Wallerian degeneration (6 mo)
DE-I6.2: Proteomic analysis of degenerating fibre tract (12 mo)
DE-I6.3: Influence of environment on proteomic profile of degenerating axons (>18 mo)

Milestones and expected result
MI-I6.1: Temporal proteomic profile of Wallerian degeneration
MI-I6.2: Impact of environmental manipulation, systemic inflammation, on proteomic profile in Wallerian degeneration

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16 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Mechanisms (Workpackage M1)

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Person-months per participant

| 3   | See H1 | 59 |

Objectives

To define the detrimental potential of CNS-infiltrating autoreactive CD8\(^{+}\) T cells using mouse models. The specific aims are:

1- To establish transgenic mouse models of CD8\(^{+}\) T-cell-mediated neuronal/axonal or glial cell damage.
2- To identify pathways and genes involved in neuronal/axonal or glial cell damage in these CD8\(^{+}\) T-cell-mediated models of neuroinflammation.

Description of work

We will generate new transgenic mice which constitutively express a neo-self antigen, the hemagglutinin (HA) of the influenza virus, in either neurons or glial cells. In addition, a transgenic system will be developed in which the expression of HA in neurons/axons or in glial cells can be induced and controlled by ingestion of doxycycline (18 months).

These HA-expressing mice will be injected with graded numbers of HA-specific cytotoxic CD8\(^{+}\) T cells originating from already established HA-specific T cell receptor-transgenic mice. We will characterize the clinical, neuropathological and immunological features of these CD8\(^{+}\) T-cell-mediated models of CNS autoimmunity. These will be compared to those of neuroinflammation induced by HA-specific CD4 T cells (available in the laboratory).

The molecular properties of CNS-infiltrating HA-specific CD8\(^{+}\) T cells compared to HA-specific, CD8\(^{+}\) T cells homing to secondary lymphoid organs will be analyzed by cDNA profiling of sorted T cell populations. The pathways and genes involved in neuronal/axonal or glial cell damage will be investigated using both neuropathological analyses and functional studies. Moreover, it becomes possible with this model, by turning off CNS HA expression, to dissociate the resulting CNS resident cell damage from the harmful T-cell inflammation.

Deliverables

DE-M1.1: Development of standardised animal models for the analysis of CD8\(^{+}\) T cell-mediated CNS tissue damage allowing pre-clinical therapeutic investigations (12 mo)

DE-M1.2: Development of transgenic mice in which the expression level of the target CNS autoantigen is controlled by administration of doxycycline (18 mo)

DE-M1.3: Identification of molecules and pathways involved in neuronal/axonal or glial cell damage (>18 mo)

Milestones\(^{17}\) and expected result

MI-M1.1: Novel information regarding the contribution of CD8 T cells in neural tissue injury.

MI-M1.2: Identification of molecular targets for protection of axons or glial cells in CD8-mediated neuroinflammation.

\(^{17}\) Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Mechanisms (Workpackage M2)

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**Objectives**
To investigate epistatic interactions in the MS-associated DR2 haplotype in humanized mouse models.

**Description of work**
We will expand our colony of transgenic mice expressing the MS associated MS MHC class II molecules DR15 and DR51 and an MS patient derived MS T cell receptor. These mice will also be backcrossed to the Rag-/- background to get mice in which the only expressed T cell receptor is the one encoded by the transgenes. We will observe how induced or spontaneous MS-like disease develops and unfolds in these mice and will characterize neuropathological changes. We will in particular test the idea, which is based on our preliminary findings that an epistatic interaction between the DR15 and 51 molecules will lead to a modified disease course in mice expressing both these MHC class molecules. We have generated DR15-MBP tetramers and will use them to quantify and characterize autoreactive DR15-MBP specific T cells in mice that express either DR15 and/or DR51.

We will ex vivo study potential differences in signalling pathways in cross-reactive T cells to understand the functional basis for how differential stimuli lead to different disease patterns. To further characterize the biophysical basis for cross-reactivity, we will express soluble T cell receptors in E. coli and characterize their binding affinity and kinetics in their interaction with soluble MHC class II-peptide complexes by using surface plasmon resonance (Biacore) measurements.

We will treat humanized MS mice using two different approaches: (i) blocking of disease-associated MHC class II molecules with the monoclonal antibody that specifically recognizes the HLA-DR15-MBP 85-99 complex. Preliminary trials have indeed shown efficacy of this antibody in mice with disease. (ii) targeting of autoreactive T cells with DR15-MBP tetramers.

**Deliverables**
DE-M2.1 Triple transgenic mice expressing human MHC class II molecules (DR15 and/or DR51) and three different human T cell receptors solely or dually restricted by these MHC molecules (18 mo).
DE-M2.2 Soluble MS-associated MHC class II molecules (18 mo)
DE-M2.3 Soluble MS-associated T cell receptors (18 mo)
DE-M2.4 DR15-MBP tetramers (12 mo)

**Milestones and expected result**
MI-M2.1 Clinical demonstration of disease modifying effect of epistatic interaction between DR15 and DR51
MI-M2.2 A functional and biophysical basis for T cell receptor cross reactivity
MI-M2.3 Clinical demonstration of disease modifying effect of treatment with anti-DR15-MBP antibody and DR15-MBP tetramers.

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18 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Mechanisms (Workpackage M3)

Objectives:
The first objective is to find out to which extent CNS lymphoid neogenesis is associated with inflammatory activity/tissue damage in MS through a detailed immunohistochemical and molecular analysis of the meninges and white matter lesions from autopic MS brains.

The second objective is to verify the contribution of CNS lymphoid neogenesis to inflammatory brain damage in mouse EAE models following intracerebral overexpression of molecules (the B-cell survival factor BAFF and the B-cell attracting chemokine CXCL13) which are implicated in B-cell follicle development and function.

Description of work:
Studies in post-mortem brain tissue from patients with different MS courses (relapsing-remitting, primary progressive and secondary progressive MS; well characterized brain tissues will be provided by P4, P7 and UK Multiple Sclerosis Tissue Bank) will involve: i) search of intrameningeal lymphoid follicles and evaluation of their association with the extent of demyelination, remyelination and neurodegeneration, using immunohistochemical techniques; ii) laser microdissection of intrathecal lymphoid follicles, intraparenchymal MS lesions and normal appearing white matter, and gene expression analysis by real-time PCR of the isolated brain tissues to identify molecules involved in lymphoid tissue formation.

Studies in preclinical models of MS will involve: i) construction, production and titration of two lentiviral vectors for the transduction of BAFF and CXCL13, respectively; ii) injection of eGFP-, BAFF- and CXCL13-expressing lentiviral vectors in the ventricular/meningeal compartment of the mouse CNS and assessment of transgene expression; iii) clinical, neuropathological and immunological assessment of disease evolution in SJL mice with PLP 139-151-induced EAE, after lentiviral vector-mediated expression of B-cell or lymphoid tissue promoting molecules in the ventricular/meningeal compartment.

Deliverables
DE-M3.1: Identification, characterization and collection of CNS germinal centers from MS brain tissue. (18 mo).
DE-M3.2: Lentiviral vectors expressing BAFF and CXCL13 (9 mo)
DE-M3.3: Animal models for the analysis of CNS compartmentalized immune responses (>18 mo)

Milestones and expected result
MI-M3.1 Knowledge about the pathogenetic role of ectopic lymphoid follicles in MS.
MI-M3.2 Collection of MS tissue which can be made available to the project participants and to other EU groups for further studies on the intrathecal immune response in MS and the identification of new therapeutic targets
MI-M3.3 Knowledge about the molecular pathways promoting the intracerebral compartmentalization of humoral immune responses.

19 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
**Subproject Mechanisms (Workpackage M4)**

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**Objectives**

To identify the mechanisms and intracellular key regulators of microglia-mediated inflammatory axonal injury.

**Description of work**

Axonal injury is the first pathological sign of most inflammatory brain diseases. In MS, axonal injury is associated with the number of macrophages and activated microglia, but the exact mechanism is not understood. Disturbance of axonal transport appears to be a major pathophysiological event in axonal injury.

*In vitro* (cultured neurons, mixed glial-neuron and organotypic cultures) of microglia/macrophage-mediated axonal injury will be established. These models aim to mimic important pathophysiological pathways involved in axonal injury in MS and other inflammatory brain diseases. We will study the effect of microglial inflammatory mediators (TNF, nitric oxide) on kinesin-mediated axonal transport of synaptic proteins (e.g. synaptophysin, beta-amyloid precursor protein) or structural cytoskeleton changes. The signaling pathways induced by microglial inflammatory mediators resulting in axonal dysfunction and injury will be studied in these *in vitro* models. The involvement of the caspase and stress kinase (JNK) signaling pathways of the TNF receptor family in axonal transport disturbance and injury will be analyzed.

Novel targets aiming to protect axons against inflammatory injury will be identified by over-expression of potentially protective genes in cultured neurons.

**Deliverables**

DE-M4.1: Knowledge on the involvement of microglial inflammatory mediators in axonal transport disturbance and axonal injury (12 mo).

DE-M4.2: Demonstration of the signaling pathways induced in axons by microglial inflammatory mediators (18 mo).

**Milestones and expected result**

MI-M4.1 Understanding the effects of microglial inflammatory mediators on axonal function and integrity.

MI-M4.2 Identification of targets for protection of axons against microglial inflammatory mediators.

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20 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.

Annex-I – “Description of work”, Page 89 of 155
Subproject Validation (Workpackage V1)

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Objectives
To define the role of TREM-2 in autoimmune CNS inflammation and test therapeutic compounds targeting the TREM-2 pathway in microglial cells in the inflamed CNS.

Description of work
In spite of its recognized role in the PLOSL dementia, the information about TREM-2/DAP12 system in the brain is still very limited. TREM-2 transcripts have been detected in human osteoclasts, human brain and mouse microglia. Furthermore, DAP-12 ko mice have been reported to be resistant to EAE induced by MOG peptide immunization. This suggests that TREM-2 may play an important role in the regulation of CNS inflammation leading to tissue damage. To study TREM-2 expression and modulate its function in \textit{in vitro} and \textit{in vivo} systems, monoclonal antibodies anti-mouse and human TREM-2, soluble human and mouse TREM-2/IgG fusion proteins, and TREM-2 small molecular weight antagonists will be tested (all these compounds will be provided by Bioxell, Milan, Italy, and their use by P1 and P19 will be regulated by a Material Transfer and Research Agreement). Using these tools, we will start to study the regulation and function of TREM-2 in inflammatory macrophages and activated microglial cells \textit{in vitro} and its expression in the brain \textit{in situ}. In addition, the effect of activating or blocking TREM-2 signalling will be evaluated during the induction of CNS inflammation and development of EAE. Clinical, neuropathological and immunological outcomes will be examined.

Deliverables
- DE-V1.1 Report on the effects of anti-TREM-2 monoclonal antibodies in EAE (18 mo)
- DE-V1.2 Report on the effect of TREM-2/IgG fusion proteins in EAE (18 mo)
- DE-V1.3 Report on TREM-2 stimulation and effects in cultured microglia (18 mo)

Milestones\textsuperscript{21} and expected result
- MI-V1.1 New knowledge on the therapeutic efficacy of compounds acting on TREM-2 pathway in EAE
- MI-V1.2 New knowledge on the role of TREM-2 in microglia activation

\textsuperscript{21} Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Validation (Workpackage V2)

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**Objectives**
To identify and target the pathways controlled by the recently identified arthritis and EAE susceptibility gene *Ncf1*

**Description of work**
a) *Ncf1* pathway analysis
Using wild type and congenic *Ncf1* mutated DA rats, we first plan to investigate the role of the *Ncf1* gene during T-cell selection in the thymus, physiological selection in the peripheral lymph nodes, immune priming in the lymph nodes and effector activation in the target tissue. Both the interacting antigen presenting cell and the responding T cell will be isolated and a functional genomic approach will be applied to find all differentially expressed products that differ between the wild type DA rat and the congenic *Ncf1* mutated DA rat. The functional genomic approach will include both microarray expression (Affymetrix) followed by real-time PCR analyses, and 2D gel analysis and masspectrometry (which will be done in cooperation with the Swegene center in Lund). The information will be analysed by bioinformaticians in our groups, interesting candidates will be analysed further by series of selected phenotypic and functional methods such as flow cytometry and immunohistochemistry.

b) Development of therapeutics targeting the *Ncf1* pathway displaying neuroprotective effects
The goal for the drug lead discovery program is to identify compounds that selectively increase the activity of the NADPH oxidase. To analyse the efficacy of new chemical structures and make comparisons with lead compounds we will use recombinant cells systems and immortalised cell lines, expressing a functional NADPH oxidase. Selected compounds will be tested in animal models of EAE. Efficacy, safety as well as ADME (administration, distribution, metabolism and excretion) in the context of various formulations will be directly addressed in these animal models.

**Deliverables**
- DE-V2.1 New genetic targets identified useful for therapy from the analysis of the *Ncf1* pathway (9 mo)
- DE-V2.2 New patented compounds with oxidant inducing activity in vivo displaying neuroprotective effects in animal models (18 mo)
- DE-V2.3 Genetic biomarkers for anti inflammatory effects of *Ncf1* targeting new drugs (18 mo)

**Milestones** and expected result
- MI-V2.1 Therapeutic efficiency of *Ncf1* targeting compounds proven in animal models of MS
- MI-V2.2 Toxicity test of new *Ncf1* targeting compounds
- MI-V2.3 First pilot clinical trial with oxidant compounds

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*Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.*

Annex-I – “Description of work”, Page 91 of 155
Subproject Validation (Workpackage V3)

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**Objectives:**
1) To develop tools that allow selective stimulation *in vivo* of TNFR2 (for enhancing neuroprotection) and inhibition of TNFR1 (for blocking inflammation).
2) As most tools will be human specific, transgenic “knock in” mice will be produced that express the two human TNF receptors to investigate *in vivo* effectiveness.

**Description of work**

On the basis of the existing human TNFR1 specific antibody H398, tools will be developed for blocking TNFR1-mediated inflammation *in vivo* (P14). To employ agents that have the capacity to fully activate TNFR2, huTNFR2-selective TNF muteins will be either covalently coupled to nanocarriers (P12 in collaboration with P20; proof of principle has been already given by P12) or a TNFR2-selective single chain TNF mutein (P12; available) will be fused to single chain antibodies (hybridomas O1 and O4 from P1, cloning and characterization by P12 and P20) directed towards galactocerebroside (oligodendrocytes) or a sulfatide (pre- and myelinating oligodendrocytes) (P12, P20).

In addition an antibody based TNFR1 specific antagonist will be developed specific for mouse TNFR1 (P12, P13, P16). Further, mouse TNFR2 specific mAbs will be tested for agonistic activity per se and after crosslinking. The model system used will be glutamate-induced excitotoxicity in primary neuronal cells (P12, P13) and oligodendrocyte cultures (P1). *In vitro* validated TNFR agonists and antagonists will be also tested *in vivo* in acute models of ischemia (P13) and MS models (P1).

Embryonic stem cells (R1 and RW4) will be electroporated with targeting constructs consisting of large parts of the mouse TNFR1 and TNFR2 gene locus, respectively. For positive/negative selection a floxed neo cassette downstream of the cDNA cassette and a terminally located herpes simplex virus TK cassette will used. Stable clones will be investigated for homologous recombination. Recombination positive clones will be injected to generate chimeric mice and breed for founder animals.

**Deliverables**

DE-V3.1: TNFR2 selective agonists (18 mo)
DE-V3.2: TNFR1 receptor antagonists (12 mo)
DE-V3.3: Recombination positive ES clones for human TNFR1 and TNFR2 (18 mo)

**Milestones**

MI-V3.1 Knowledge about therapeutic potential of TNFR specific antibodies derived from neuronal culture systems
MI-V3.2 Available constructs and ES cells for production of human TNFR knock-in mice

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23 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Validation (Workpackage V4)

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Objectives:
1) To validate the role of the neuronal TNFR1 core signaling scaffold, and key intracellular signaling components in neuroprotection during the development of EAE and upon ischemic and excitotoxic injury.
2) To use this information to develop cell-penetrating small molecule drugs that will enhance neuron protection and to evaluate their potential as novel neuroprotective agents in vivo.

Description of work
The critical requirement of the neuronal TNFR1 core signaling scaffold for the protection of neurons from delayed (apoptotic) death mechanisms triggered by injury has been identified using mice deficient in the TNFR in experimental models of ischemic stroke in vitro and in vivo. Partner P14 has recently identified a novel TNF-inducible, caspase-dependent and NFκB-activating pathway that is responsible for this TNFR1-mediated neuroprotection. In this workpackage we propose:
1) To validate the role of identified TNFR1 signaling mediators in NFκB activation and neuroprotection in primary wild-type mouse neurons (cortical and hippocampal) using established in vitro models of neuron injury (glucose deprivation, glucose/oxygen deprivation, kainic acid excitotoxicity). Mediators to be investigated include the proximal TNFR1 signalling components FADD, caspase 8, long isoform of FLIP (FLIP L), RIP and TRAF2, and the specific downstream mediator p50/p65 NFκB. Protein function will be verified using gene knockdown and protein inhibition techniques with lentiviral delivery of siRNA and dominant negative protein forms respectively, and by overexpression of wild-type or mutant proteins fused to the TAT sequence of HIV for efficient intracellular delivery.
2) To use this information to rationally design peptides and small molecules with neuroprotective function.

To functionally evaluate cell-penetrating peptides as neuroprotective agents in vitro in primary neurons under conditions of apoptosis (glucose deprivation, glucose/oxygen deprivation, kainic acid excitotoxicity), and in vivo in mouse models for MS (MOG-EAE in C57BL/6 mice, Tg6074 TNF transgenic mice), cerebral stroke (middle cerebral artery occlusion, pMCAO) and kainic acid epileptic seizures.

Deliverables
DE-V4.1 Detailed knowledge of neuronal TNFR1-mediated neuroprotective signaling (12 mo)
DE-V4.2 Identification of cross-talk with neurodegenerative pathways (e.g. JNK-mediated, glutamate) and determination of the mechanism of neuroprotection (12 mo)
DE-V4.3 Development and screening of cell-penetrating peptides designed to have neuroprotective function (18 mo)
DE-V4.4 Combination therapies of peptides with TNFR agonists and antagonists (developed in V3) and other relevant neuroprotective compounds (e.g. JNK inhibitors) (18 mo)

Milestones and expected result
MI-V4.1 Validation of the role of key TNFR1 signaling components in providing neuroprotection
MI-V4.2 Design and functional evaluation of cell-penetrating peptides based on neuroprotective TNFR1 signaling components as therapeutic candidates.

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24 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Validation (Workpackage V5)

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**Objectives:** To determine the efficacy for axonal protection of 1) sodium channel blocking agents that are either selective for persistent sodium currents, or for different sub-types of sodium channels, and 2) drugs that inhibit the reverse mode of operation of the sodium-calcium exchanger.

**Description of work**

Sodium channel blocking drugs will include lamotrigine, flecainide, carbamazepine, phenytoin and 202W92 and other proprietary drugs (PD). Exchange blockers will include bepridil and PD. PD will come from our existing collaborative agreements with several European pharmacological companies, including Gedeon Richter, Hungary, GlaxoSmithKline, UK, and UCB, Belgium.

We will explore the efficacy of the different drugs and different approaches using our proven, stepwise screening progression through a) *in vitro* (Smith et al., 2001; Ann Neurol., 49:470-476), b) semi *in vivo* (Kapoor et al., 2003; Ann Neurol., 53: 174), and c) fully *in vivo* models (Bechtold et al., 2004; Ann Neurol. 55:607). In our initial experiments we will expose isolated dorsal and ventral rat spinal roots *in vitro* to damaging (inflammatory) concentrations of nitric oxide (NO) generated by the NO donor DETA NONOate. We will concurrently apply drugs to try to protect the axons from degeneration, and compare the efficacy of the drugs using electrophysiological and morphological (immunohistochemistry and high resolution microscopy) methods. The best drugs will then be advanced for examination in our semi *in vivo* experiments in which the drugs will be directly applied to exposed dorsal roots having an intact physiological vasculature in anaesthetised rats. The roots will be concurrently exposed to NO (DETA NONOate) and sustained impulse activity (to mimic events in an MS lesion): we have previously shown that in the absence of pharmacological protection this regimen causes axonal degeneration. The optimum drugs will be identified by histological examination of axonal integrity and these will be advanced for study in later years using in *vivo* models of MS, including MOG-induced EAE. The best drugs identified in EAE will subsequently be advanced for clinical trial in MS patients.

**Deliverables.**

DE-V5.1 Sodium channel blockers that are most effective in protecting axons from degeneration in *in vitro* and semi *in vivo* models (18 mo)

DE-V5.2 Drugs that inhibit the reverse mode of operation of the sodium-calcium exchanger and are most effective in protecting axons from degeneration in *in vitro* and semi *in vivo* models (18 mo)

**Milestones** and expected result

MI-V5.1 Identification of the best sodium channels blockers to be tested for their neuroprotective efficacy in *in vivo* experiments in EAE-affected mice and rats.

MI-V5.2 Identification of the best inhibitors of the reverse mode of operation of the sodium-calcium exchanger to be tested for their neuroprotective efficacy in *in vivo* experiments in EAE-affected mice and rats.

MI-V5.3 Identification of drugs suitable for examination in clinical trials in MS.

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25 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Objectives.

To develop novel therapeutic approaches for neuroprotection targeting glutamate and calcium channel dysfunction. Furthermore, the aim is to dissect how glutamate and calcium channel dysfunction cause neuronal/axonal damage within the inflamed EAE spinal cord in MS.

Description of work.

We previously demonstrated that both pharmacological intervention in the glutamatergic pathway and genetic manipulation of N-type calcium channels reduces neuronal and axonal damage in animal models of MS and there is growing consensus regarding the importance of glutamate and calcium in axonal damage. Several MS models, including acute (MBP-Lewis rat) and chronic (PLP-SJL/J mice; MOG-C57Bl6 mice) EAE, are routinely employed in P18 (EP) laboratories, and these models will be augmented with models used by other participants. The models and approach described in workpackage V5 will be employed, and, in collaboration with colleagues in University College London, spinal cord slices from control and EAE animals will be loaded with fluorescent calcium indicators to enable real time analysis of spontaneous activity and intercellular communication in both neuronal and glial compartments.

A panel of pharmacological tools (glutamate receptor antagonists, modulators and uptake inhibitors, sodium-calcium exchange blockers and calcium channel blockers) are already available to us for use alone and in conjunction with immunomodulatory agents (IFN-beta, NO scavengers, TNF neutralising agents, etc.) to identify the sequence and effectors of neuronal damage in the spinal cord.

Deliverables.

DE-V6.1 Establishment of various EAE models to be utilised for in vivo screening (6 mo)
DE-V6.2 Establishment of calcium imaging methods in ex-vivo spinal cord slice (12 mo)
DE-V6.3 Definition of parameters of glutamatergic/ion channel signalling in axonal degeneration in EAE (18 mo)

Milestones and expected result.

MI-V6.1 Determination of the relative contributions of glutamate receptors vs. Na⁺/Ca²⁺ exchange to neurodegeneration in EAE.
MI-V6.2 Identification of pharmacological agents modulating glutamate receptors and calcium channels for use in in vitro and in vivo neuroprotection models.

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26 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Validation (Workpackage V7)

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**Objectives**
To identify safe and effective strategies to protect neurons and axons from EAE-associated degeneration based on the development of combination therapies consisting in anti-inflammatory/immunomodulatory and anti-apoptotic neuroprotective approaches.

**Description of work**
Step I: Prelabeling of retinal ganglion cells (RGCs) by stereotactic injection of a fluorescent dye (Fluorogold) into both superior colliculi.
Step II: Clinical and electrophysiological effects of the fusion proteins TAT-Bcl-2 and TAT-GDNF, flupirtine (a Bcl-2 inducer), simvastatin (a potential PI-3-kinase activator), or minocycline (a potential activator of MAPKs and PI-3-kinase) in MOG-EAE in BN rats. All treatment groups (+ respective vehicle-treated control groups) will be scored and weighted daily; assessment of visual evoked potentials and electroretinograms will be performed at the day of disease onset as well as on day 8 of MOG-EAE (end of study).
Step III: Histopathological analysis of brain, spinal cord and optic nerves in each animal; determination of retinal ganglion cell (RGC) counts, investigation of potential intracellular neuroprotective pathways in RGCs. These studies will involve: i) Preparation, cutting, histopathological staining of brains, spinal cord and optic nerves (Luxol fast blue to assess the extent of demyelination, Bielschowsky silver impregnation for axonal densities, APP for acute axonal damage, hematoxylin-eosin for inflammatory infiltration), data analysis; ii) whole mount preparation of retinas and RGC counting; iii) immunohistochemistry and Western Blot analysis of intracellular neuroprotective signal transduction pathways in RGCs.

**Deliverables**
DV7.1 EAE models with strong neurodegenerative aspects for therapeutic treatment evaluation (18 mo)
DV7.2 In vivo electrophysiology in rodents (evoked potentials) (18 mo)
DV7.3 Fusion proteins for transduction of anti-apoptotic proteins (6 mo)

**Milestones**

27 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Horizontal Integration (Workpackage H1)

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Objectives

1) To organize a Neuropromise Neuropathology Reference Center (NNRC) which will provide neuropathological support for the workpackages summarized under the subprojects “Identification”, “Mechanisms” and “Validation”;
2) To optimise technical procedures and personnel training.

Description of work

1) Provision of extensively characterized human autopsy and biopsy tissue for expression studies of newly identified target genes for MS susceptibility and phenotype. Partners P2b and P5 have collected a large repository of autopsy and biopsy tissue from more than 200 MS patients. This material is extensively characterized for inflammation, demyelination, remyelination and axonal/neuronal injury. A large collection of autopsy material of normal brain and other inflammatory brain diseases is also available. Immunohistochemical and in situ hybridisation analyses will be performed in relation to specific aspects of the lesions, like inflammation, active demyelination, active axonal/neuronal injury and remyelination.
2) Creation of a genomic DNA bank from archival biopsy and autopsy tissue, which is extensively characterized as described above. Newly identified disease-related gene polymorphisms will be analysed in this material by PCR-technology and directly related to specific pathological alterations (as above).
3) Pathological analysis of CNS tissue from animal models utilized in subprojects Identification, Mechanisms and Validation, in direct cooperation with the involved partners.
4) Training and education of the NeuroproMiSe consortium partners in neuropathological analysis of experimental material, including conventional histology, immunohistochemistry, in situ hybridisation, confocal laser microscopy and electron microscopy. These training activities will be performed on an exchange basis, giving collaborators of the partners the opportunity to learn neuropathological techniques under the guidance of partners P4 and P7 in the course of short term laboratory visits (see also T1).

Deliverables

DE-H1.1 Quantitative characterization of human brain lesions in biopsy and autopsy material in relation to inflammation, demyelination, remyelination and axonal injury (>18 mo)
DE-H1.2 DNA isolation from archival biopsy and autopsy tissue (18 mo)
DE-H1.3 Expression studies of newly identified targets in human tissue in relation to specific aspects of pathology (>18 mo)
DE-H1.4 Neuropathological analysis of experimental models (> 18 mo)
**Milestones and expected result**

MI-H1.1 Validation of the expression of new potential targets within multiple sclerosis lesions in comparison to controls and its correlation with specific pathological phenotypes of the disease.

MI-H1.2 Identification of new mechanisms of neuronal injury in inflammatory brain diseases by pathological analysis of disease models.

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28 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Horizontal Integration (Workpackage H2)

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Objectives

1) To provide adequate support for high throughput genomic and proteomic studies to be performed by the consortium participants

2) To organise genome-wide information collected from heterogeneous neuroimmune-mediated diseases into an artificial neural network and thereby create an in silico simulation of the CNS inflammatory response and disease-related pathways.

Description of work

NeuroproMiSe Genomics Reference Center (ULUND)

The technical platform includes animal-based genomic technology and analysis. It has the capacity to provide pathway analysis through functional genomic methodology such as expression array as well as possibilities to identify genetic polymorphisms in complex genetics of animal model diseases. Furthermore, it provides the structure to identify new pathways through microarray technology, bioinformatics, and statistical methods to calculate the effects and to follow up results using biological experiments. Data from expression studies will be integrated in a data base to allow a genotype-phenotype relation and promote collaborative studies addressing specific genes. A genotype-phenotype relation database developed by P17, and utilized by P3 and P17, will be open to the consortium.

NeuroproMiSe Proteomics Reference Center (USOU)

The technical platform offers protein expression studies, 2-D-electrophoresis and structural analyses of material obtained from animal models or humans.

NeuroproMiSe Artificial Neural Network (HPI)

Genomic platforms will be further developed in this project to provide an artificial neural network for linking gene expression changes to CNS disease. Using well-defined mouse models for CNS inflammatory and neurodegenerative diseases (MOG-EAE, Tg6074 TNF transgenics, TgAPP transgenics and pMCAO, all in a C57BL/6 genetic background), the P14 team has generated detailed gene expression data using a 27K element mouse cDNA microarray. Duplicated data sets obtained from diseased and wild-type control brain, at multiple time points through disease initiation and development, have been normalized and will be integrated into a 3-dimensional neural network-type algorithm which will robustly classify gene expression changes, e.g. into disease-common and disease-specific categories. This algorithm will be further developed with the aim of producing a sensitive and versatile, user-friendly bench-top tool which will be of use for the identification of new therapeutic targets, delineation of disease mechanisms and pathways, and for rapid assessment of the outcome of therapeutic regimens in the different models utilized by the project participants. Work will involve:

1) validation of the algorithm by verification of changes in expression of numerous selected genes using RealTimePCR and in situ hybridisation (in collaboration with P5);

2) strengthening of algorithm sensitivity using gene expression data derived from blinded disease/tissue-related and unrelated samples;

3) testing of the algorithm effectivity within this consortium for evaluation of the outcome of experimental therapeutic regimens.
**Deliverables**

DE-H2.1 Gene expression profiles of animal models of disease (>18 mo)
DE-H2.2 Protein expression profiles of animal models of disease (>18 mo)
DE-H2.3 Fully validated computer-based algorithm representing gene changes associated with 4 independent mouse models representing human MS, Alzheimer’s disease and cerebral stroke (18 mo)

**Milestones29 and expected result**

MI-H2.1 Genomics support for the identification of disease-associated genes and molecular pathways
MI-H2.2 Proteomics support for the identification of disease-associated proteins
MI-H2.3 Completion of CNS inflammation algorithm validation using independent gene expression data.
MI-H3.4 Fine-tuning of algorithm stringency using blinded gene expression data sets obtained from independent models and tissues
MI-H3.5 Application of algorithm to the evaluation of data sets derived from the present project (e.g. for assessing the outcome of experimental therapeutic regimens)

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29 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Training (Workpackage T1)

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Objectives
To set up an educational training programme to promote the synergies of the cooperating scientific groups.
To attract young scientists and support them through the Marie Curie Fellowships
To train the scientific personnel in aspects related to IPR, result exploitation, ethical and society issues

Description of work
The training programme will be established in accord with the principal tasks of the project. The programme will be presented in the NeuroproMiSe website. For each course, coordinator, contents, duration, and number of participants will be defined.
Scientific training for young scientists will be performed in topics related to horizontal integration activities (see WPs H1, H2).
Periodical search for Marie Curie Fellowships will be performed.
Thematic training will be performed on: exploitation of results and patents issues, ethical issues (particularly animal experimentation) and gender issues.

Deliverables
DE-T1.1 Programme of exchange scientific courses among the partners (10 mo)
DE-T1.2 Calls for candidates for Marie Curie Fellowships (18 mo)
DE-T1.3 Courses on functional genomics (18 mo)
DE-T1.4 Short term personnel exchange for training in Neuropathology (18 mo)

Milestones and expected result
MI-T1.1 Training of young scientists
MI-T1.2 Attraction of young scientists
MI-T1.3 Knowledge dissemination on result exploitation and IPR, ethical and society issues

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30 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
Subproject Training (Workpackage T2)

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**Objectives**

To organize workshops to communicate the scientific results developed by each participant group and to train other researchers and potential stakeholders on the knowledge, methods, technologies, as well as new potential products generated in the IP and their use.

**Description of work**

A series of thematic workshops covering the major topics addressed in the project will be programmed and circulated through the NeuroproMiSe web-site.

Organization of specific technical meetings on selected issues relevant to the project.

Coordination with the activities of other International and EU training initiatives and programmes (International Graduate School for MD-PhD in Neurosciences associated with Göttingen Universit, ESNI, EFNS).

Continuous updating on EU and International Courses, Workshops and Meetings related to issues relevant for the project, through the NeuroproMiSe website.

The Subproject coordinators and workpackage leaders will deliver lectures in seminars and workshops.

**Deliverables**

DE-T2.1 Programme of thematic workshops and technical meetings (12 mo)

DE-T2.2 Workshop on ethical issues (12 mo)

DE-T2.3 Workshop on the following topics (18 mo):

- Genetics of MS
- Mechanisms of neuronal degeneration and protection
- Novel concepts for the therapy of neuroimmune diseases
- Development of protein and peptide drugs for neuroinflammation

**Milestones**

31 **Milestones and expected result**

MI-T2.1 Scientific knowledge dissemination

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31 Milestones are control points at which decisions are needed; for example concerning which of several technologies will be adopted as the basis for the next phase of the project.
## Subproject Management (Workpackage MAN)

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### Objectives

**Coordination of Science, Business and Administrative Management**

- To coordinate all the project activities within the consortium and with respect to the European Commission.
- To coordinate all activities required for scientific implementation, IP protection and exploitation of NeuroproMiSe results.
- To ensure effective interaction among the Subprojects and Workpackages.
- To evaluate and protect the know-how resulting from the project activities and consequent industrial exploitation.
- To oversee training activities and establish dissemination plans.
- To monitor both science- and society-related issues, including community involvement, ethical and gender issues related to the project activities.

### Description of work

- To control the initial working phases and activities, integrate them properly, and monitor their development according to the specific objectives and timelines.
- Daily management of the scientific activities within the consortium and to the EC.
- Daily management of the training and dissemination activities.
- Daily management of the administrative activities within the consortium and to the EC.
- Daily management of the business activities within the consortium and to the EC.
- Development of an Internet website in which all relevant information and a discussion forum will be made available to the members of the consortium and for dissemination of the consortium activities and results to the public.

### Deliverables

- DE-MAN.1 First Steering Committee meeting (1 mo)
- DE-MAN.2 Project presentation (1 mo)
- DE-MAN.3 Development, implementation and updating of the NeuroproMiSe web-site (6 mo)
- DE-MAN.4 Steering Committee meeting (6 mo)
- DE-MAN.5 Steering Committee meeting (12 mo)
- DE-MAN.6 General Assembly meeting (12 mo)
- DE-MAN.7 Submission of the Annual Progress Report to the EC (12 mo)
- DE-MAN.8 Submission of the next 18 month Detailed Implementation Plan to the EC (18 mo)
- DE-MAN.9 Steering Committee Meeting (18 mo)
- DE-MAN.10 Presentation of abstracts to national and international meetings and submission of publications to peer-reviewed journals (18 mo)
- DE-MAN.11 Intellectual property actions and technology transfer activities (18 mo)
- DE-MAN.12 Dissemination plan (12 mo)
- DE-MAN.13 Gender action plan (12 mo)
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<td>MI-MAN.3 NeuroproMiSe website</td>
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<td>MI-MAN.5 Implementation plan for the next 18 months</td>
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\(^{32}\) Specify efforts in person months
9 PROJECT RESOURCES AND BUDGET OVERVIEW
### 9.1 IP PROJECT EFFORT FORM 1 -FULL DURATION OF PROJECT (TOTAL WORKFORCE: EU FUNDED PLUS NON-EU FUNDED)

**IP Effort Form - Indicative efforts for full duration of project**

*Project number (acronym): 018637 (NEUROPROMISE)*

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</table>
### 9.3 OVERALL BUDGET FOR FULL DURATION OF THE PROJECT (Forms A3.1 & A3.2 FROM CPFS)

**FORM A3.2**

#### Annex-I – “Description of work”, Page 111 of 155
### Contract Preparation Forms

**Integrated Project**

**NeuroproMiSe**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Organisation</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (whole duration of the project)</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts</th>
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<tbody>
<tr>
<td>6 INSERM FCF</td>
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<td>12,000.00</td>
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<td>18,000.00</td>
<td>12,000.00</td>
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<td>of which subcontracting</td>
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<td>10,000.00</td>
<td>578,800.00</td>
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<tr>
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<td>18,000.00</td>
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<td>537,340.00</td>
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## Contract Preparation Forms

### Integrated Project

**A3.1**

### Proposal Acronym: NeuroproMiSe

#### Annex I – “Description of work”, Page 113 of 155

<table>
<thead>
<tr>
<th>Participant</th>
<th>Organisation short name</th>
<th>Organisation name</th>
<th>Cost model used</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts</th>
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<tbody>
<tr>
<td>11</td>
<td>KCL</td>
<td>AC</td>
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<td></td>
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<td>of which subcontracting</td>
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<tr>
<td>12</td>
<td>USTUTT</td>
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<td>650,800.00</td>
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<td></td>
<td>of which subcontracting</td>
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<td>90,200.00</td>
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</table>
### Financial information - whole duration of the project

<table>
<thead>
<tr>
<th>Participant</th>
<th>Organisation short name</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (whole duration of the project)</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 ARX</td>
<td>FC</td>
<td></td>
<td>Direct Costs (a) 711,000.00, of which subcontracting 65,000.00, Indirect costs (b) 513,000.00</td>
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<td>726,000.00</td>
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<tr>
<td>18 ELL</td>
<td>FCF</td>
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<td>Direct Costs (a) 1,297,000.00, of which subcontracting , Indirect costs (b) 259,400.00</td>
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<td>1,577,400.00</td>
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<tr>
<td>19 UNI-BONN</td>
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<td></td>
<td>Direct Costs (a) 590,200.00, of which subcontracting , Indirect costs (b) 118,040.00</td>
<td>RTD or innovation related activities (1) 0.00, Demonstration activities (2) 0.00, Training activities (3) 15,000.00, Consortium Management activities (4) 0.00</td>
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<tr>
<td>20 DIREVO</td>
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<td>Direct Costs (a) 322,500.00, of which subcontracting , Indirect costs (b) 66,500.00</td>
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<td><strong>TOTAL</strong></td>
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<td>Eligible costs 13,167,841.25, Requested EC contribution 10,409,846.43</td>
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<td><strong>11,399,997.43</strong></td>
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## FORM A3.2 Estimated breakdown of the EC contribution per reporting period

### Contract Preparation Forms

**Integrated Project**

**NeuroproMiSe**

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<th>Proposal Number</th>
<th>Proposal Acronym</th>
<th>NeuroproMiSe</th>
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<td>2,289,024.36</td>
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<td>Reporting Period 4</td>
<td>M37 - M48</td>
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<tr>
<td>Reporting Period 5</td>
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9.4 Budget for the first 18 months (Form A3.3 from CPFs)

### Financial information - first 18 months of the project

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<th>Participant</th>
<th>Organisation</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (first 18 months of the project)</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts</th>
<th>Month 1-18</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>Direct Costs (a) 154,560.00, Indirect Costs (b) 30,912.00, Total eligible costs (a)+(b) 185,472.00</td>
<td>RTD or innovation related activities (1) Month 1-18 154,560.00, Demonstration activities (2) Month 1-18 0.00, Training activities (3) Month 1-18 1,200.00, Consortium Management activities (4) Month 1-18 107,500.00</td>
<td>219,264.00</td>
<td>219,264.00</td>
</tr>
<tr>
<td>2</td>
<td>UKG-GOE</td>
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<td>Direct Costs (a) 182,720.00, Indirect Costs (b) 36,544.00, Total eligible costs (a)+(b) 219,264.00</td>
<td>RTD or innovation related activities (1) Month 1-18 182,720.00, Demonstration activities (2) Month 1-18 0.00, Training activities (3) Month 1-18 12,000.00, Consortium Management activities (4) Month 1-18 12,000.00</td>
<td>219,264.00</td>
<td>219,264.00</td>
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<tr>
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<td>ULUND</td>
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<td>204,000.00</td>
<td>204,000.00</td>
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<tr>
<td>4</td>
<td>MRC</td>
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<td>Direct Costs (a) 229,416.00, Indirect Costs (b) 70,450.00, Total eligible costs (a)+(b) 300,666.00</td>
<td>RTD or innovation related activities (1) Month 1-18 229,416.00, Demonstration activities (2) Month 1-18 0.00, Training activities (3) Month 1-18 4,380.00, Consortium Management activities (4) Month 1-18 4,380.00</td>
<td>204,000.00</td>
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</tr>
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</table>

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Annex-I – “Description of work”, Page 116 of 155
## Financial information - first 12 months of the project

<table>
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<th>Estimated eligible costs and requested EC contribution (first 12 months of the project)</th>
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<th>Demonstration activities (2) Month 1-18</th>
<th>Training activities (3) Month 1-18</th>
<th>Consortium Management activities (4) Month 1-18</th>
<th>Total (5)=(1)+(2)+(3)+(4) Month 1-18</th>
<th>Total receipts Month 1-18</th>
</tr>
</thead>
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<td>0.0</td>
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<tr>
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<td>16,000.00</td>
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<td>0.0</td>
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<td>of which subcontracting</td>
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<td>3,800.00</td>
<td>251,960.00</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Requested EC contribution</td>
<td>242,640.00</td>
<td>0.0</td>
<td>5,520.00</td>
<td>0.0</td>
<td>3,600.00</td>
<td>251,960.00</td>
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</table>
### Contract Preparation Forms

#### Integrated Project

**A3.3**

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**Financial information - first 18 months of the project**

<table>
<thead>
<tr>
<th>Participant</th>
<th>Organisation short name</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (first 18 months of the project)</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts (5)=(1)+(2)+(3)+(4)</th>
<th>Total receipts Month 1-18</th>
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<td>9 NPHI AC</td>
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<td></td>
<td>Direct Costs (b)</td>
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</tr>
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</table>

Annex-I – “Description of work”, Page 118 of 155
## Contract Preparation Forms

### Integrated Project

**A3.3**

Please use as many copies of form A3.3 as necessary for the number of partners.

<table>
<thead>
<tr>
<th>Proposal Number</th>
<th>018637</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposal Acronym</td>
<td>NeuroproMiSe</td>
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</tbody>
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### Financial information - first 18 months of the project

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<thead>
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<th>Participant</th>
<th>Organisation short name</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (first 18 months of the project)</th>
<th>Costs and EC contribution per type of activities</th>
<th>Total receipts Month 1-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 USGR</td>
<td>AC</td>
<td></td>
<td>Direct Costs (a) 98,400.00 of which subcontracting 0.00 Indirect costs (b) 19,680.00</td>
<td>Total eligible costs (a)+(b) 118,080.00 Requested EC contribution 118,080.00</td>
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<td>14 HPI</td>
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<td></td>
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<td>Total eligible costs (a)+(b) 156,760.00 Requested EC contribution 156,760.00</td>
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<td>FCF</td>
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<td>FC</td>
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</table>

Annex-I – “Description of work”, Page 119 of 155
## A3.3

### Financial information - first 18 months of the project

<table>
<thead>
<tr>
<th>Participant</th>
<th>Organisation</th>
<th>Cost model used</th>
<th>Estimated eligible costs and requested EC contribution (first 18 months of the project)</th>
<th>RTD or innovation related activities (1)</th>
<th>Demonstration activities (2)</th>
<th>Training activities (3)</th>
<th>Consortium Management activities (4)</th>
<th>Total (5) = (1) + (2) + (3) + (4)</th>
<th>Total receipts Month 1-18</th>
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<td>15,000.00</td>
<td>15,000.00</td>
<td>408,100.00</td>
<td>487,920.00</td>
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<td></td>
<td>Requested EC contribution 233,460.00</td>
<td>8,000.00</td>
<td>15,000.00</td>
<td>254,460.00</td>
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<td></td>
</tr>
<tr>
<td>19 UNI-BONN</td>
<td>AC</td>
<td>Eligible costs</td>
<td>Direct Costs (a) 181,700.00, of which subcontracting 0.00, Indirect costs (b) 36,340.00, Total eligible costs (a)+(b) 218,040.00</td>
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<td>15,000.00</td>
<td>204,800.00</td>
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<td></td>
<td></td>
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<td>245,760.00</td>
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<td>20 DIREVO</td>
<td>FCF</td>
<td>Eligible costs</td>
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<td>3,000.00</td>
<td>122,700.00</td>
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<td>Eligible costs</td>
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<td></td>
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9.5 IP MANAGEMENT LEVEL JUSTIFICATION OF RESOURCES AND BUDGET

The total project resources and efforts are summarized in Table B.7.2.1, at the end of this section.

The total budget for the project is 31.082.276,00 €, of which 17.157.684,00 € in personnel and infrastructural resources are provided directly by the contractors, and 13.924.592,00 € are total costs for which an EU contribution of 11.399.997,00 € is being requested as a grant.

The specific costs are explained as follows:

**Personnel Costs**

For the tasks described in sections 6 and 8, the consortium requests 20 positions under the AC model and 6 positions under the FC or FCF model, totalling 6.811.703,00 € of direct personnel costs for the five year period. This represents only a fraction of the total work force implemented in the Integrated Project NeuroproMiSe. With an average contribution of about 50% to the specific project tasks, this comprises a work power of approximately 90 persons or 11.898.757,00 € on direct personnel costs (excluding overheads), with an average of 4,3 persons per participant. In addition, a substantial input will be provided from resources of participants in general technical infrastructure and specific equipment, all essential for the execution of the tasks. These personal costs will cover the training activities.

Additional to the costs for scientific personnel, costs equivalent to 2 positions (one full-time and one part-time) will be needed for management tasks. These positions will be allocated to the coordinator to support him in the management of the consortium. The other personal cost for management is allocated to the co-coordinator (5 months).

**Equipment**

Total costs for equipment amount to 120.000,00 €, and include: a laser capture microdissection system equipped with fluorescence microscope (100.000,00 €) requested by P1/ISS and necessary for selection and isolation of single cells, groups of cells and specific anatomical structures from mouse and human brain tissue; a monitor apparatus for prolonged anesthesia required for electrophysiological studies, computer based data acquisition system and operating microscope requested by P11/KCL (for a total of 20.000,00 €).

**Consumables for the scientific subprojects**

The scientific and technological work described creates substantial costs for SNP genotyping, microarrays, proteomics, reagents (biological, chemical), cell culture materials, biosynthesis of peptide and protein drugs, animals, pharmacological, physiological and pathological studies. These costs are summarized under consumables and animal studies, for a total of 3.171.375 € for the 5-year period.

**Training costs**

A substantial part of the training activities of the consortium are devoted to horizontal distribution of technological, methodological expertise among the participants and to researchers outside. For the planned experimental courses, specific costs will arise for organization and consumables. Eight technical workshops are planned, each with a calculated cost of 12.000,00 €, totalling 96.000, 00 € for the five year period.

For the organization of training workshops other costs will be necessary. We estimate 15.000,00 € each for the planned three internal workshops and 30.000,00 € for the last report symposium with external speakers and participants.

**Travel costs**

Travel costs will be necessary for communication within the consortium and with the scientific community in general, and specifically for training and management activities. We estimate travel costs to amount to 288.000,00 €. We expect these costs to allow each participant to cover the following activities:

- visit at least one partner per year for collaboration purposes;
- attend to one international scientific meeting;
- participate in training courses;
- attend the annual General Assembly meeting;
- attend the biannual SC meetings (SC members only).

**Computing**
For computing, a total of 21,600 € is requested.

**Patenting costs** are requested by P7, P11, P18 and P19, accounting for a total of 60,000,00 €.

**Management costs**
Besides personnel (see above), the management costs include the costs for the NeuroproMiSe web-site, travel costs for the members of the Steering Committee and Advisory Board, organization of the Steering Committees, the annual General Assembly and the Final Report Workshop. Together with subcontracting (see below), the management costs account for 6.55% of the EU contribution.

**Subcontracting**
To support the management activities, a Professional Accounting Firm will be retained, for the whole duration of the project, as a Subcontractor of the Coordinator, to coordinate and oversee the overall financial and administrative management of the Consortium. The total amount expected to be devoted to this task amounts to 15,000,00€ Euro per year (total 75,000,00€).
The cost for audit certificates for some participants, to be subcontracted, is about 2,000,00 € per year, for a total of 140,000,00 € for the whole project duration.
Participant P17/ARX requests subcontracting to three companies for the development of new therapeutic compounds, in particular for chemical synthesis, biodistribution studies, and preclinical safety and toxicology studies, for a total amount of 85,000,00 €.

Participants operating under the AC model (P1, P2a/P2b UK-GOE, P3, P5, P7, P8, P9, P10, P11, P12, P13, P14, P19) request an overhead of 20% to their above specified costs; participants P4, P6, P16, P17, P18, and P20 operate under FC/FCF conditions and request a 50% contribution.
Table 9.5.1 Combined project resources and efforts

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<th>Eligible Costs</th>
<th>ELIGIBLE COSTS (&quot;EC&quot;)</th>
<th>NonEC Costs</th>
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<td>RTD</td>
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<td>Av. Cost per PM</td>
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<tr>
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<tr>
<td>Equipment</td>
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<tr>
<td>Travel &amp; Lodging</td>
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<td>Consumables</td>
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<td>Animal studies</td>
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<td>Patenting costs</td>
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<tr>
<td>Other (WEB, SC meetings, final workshop)</td>
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<td><strong>Subtotal</strong></td>
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<tr>
<td>Subcontracting</td>
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<td><strong>Total Direct</strong></td>
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10 ETHICAL ISSUES

In this section, we have integrated the ethical issues included in the proposal, those requested by the EC as additional information, and the requirements/recommendations indicated in the Ethical review report (marked in yellow).

Identification of countries where research will be carried out (participants are indicated in brackets):
Animal experiments: Italy (P1); Germany (P2a, P7, P19); Sweden (P3, P8, P17); UK (P4, P10, P11, P18); France (P6); Finland (P9); The Netherlands (P13); Greece (P14).
Genetic and neuropathological studies with human biological material: Germany (P2b); Austria (P5); France (P6); Sweden (P8); Finland (P9).
Work with genetically modified organisms: Italy (P1); Germany (P2a, P7, P12, P19); Sweden (P3); France (P6); UK (P6); Finland (P9); The Netherlands (P13); Greece (P14).

The NeuroproMiSe consortium will address the ethical, legal, social and safety issues related to research with human biological samples, use of personal data and genetic information, animal experiments, generation and use of genetically modified organisms. All partners involved in these activities have a long standing expertise in these matters and are registered laboratories holding permissions for experimental conditions as planned within the NeuroproMiSe project.

We confirm that all participants in the NeuroproMiSe project will conform to current legislation and regulations in EU and in the countries where the research will be carried out (see below). We further confirm that each partner has already obtained the necessary authorizations to carry out the studies related to this proposal (see below), and that in case additional experiments have to be carried out, not yet comprised within the existing permissions, we seek to obtain these permissions from the respective authorities and will not begin experimentation before approval is obtained and the Commission has been informed.

Clinical trials: Clinical trials are not planned. In case of an upcoming clinical trial, special approval is required according to the relevant national regulations. Each study requires prior approval by the local ethics committee of each of the involved clinical partners.

Ethical issues form

a) Specify if your project involves:

<table>
<thead>
<tr>
<th>Does your proposed research involve:</th>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
<tbody>
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<td>• Human beings</td>
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<td>X</td>
</tr>
<tr>
<td>Persons not able to give consent</td>
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<tr>
<td>Children</td>
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<td>X</td>
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<tr>
<td>Adult healthy volunteers</td>
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<td>X</td>
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<tr>
<td>• Human embryos</td>
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<td>X</td>
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<tr>
<td>• Human biological samples</td>
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<td>Human embryonic stem cells</td>
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<td>X</td>
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<tr>
<td>Human foetal tissue/cells</td>
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<td>• Other personal data</td>
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<tr>
<td>Sensitive data about health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction</td>
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<td></td>
</tr>
<tr>
<td>• Animals (any species)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Non-human primates</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
Transgenic small laboratory animals | X |
---|---|
Transgenic farm animals | X |
Cloning of farm animals | X |
- Developing countries (e.g. clinical trials, use of human and animal genetic resources…) | X |
- Dual use | X |

The Coordinator and the Steering Committee confirm that the proposed research does not involve:
research activity aiming at human cloning for reproductive purposes,
research activity intended to modify the genetic heritage of human beings which could make such changes heritable,
research activities intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer.

**Research involving human beings and their tissues**
All studies utilizing human material will be conducted only after approval of the local ethical committees and adhering strictly to their regulations. These are fully compliant with international laws and policies governing the use of human materials and clinical studies:
- The Helsinki declaration in its latest version
- The Charter of Fundamental Rights of the EU (2000/C 364/01)
- The European directive 95/46 EEC on the protection of individuals with regard to the processing of personal data and on the free movement of such data
- The Universal Declaration on the Human Genome and Human Rights, adopted unanimously and by acclamation by the General Conference of UNESCO at its 29th session on 11 November 1997.
- The consortium is aware of the opinion given to the European Commission by the “European Group on Ethics in Science and New Technologies” on “The patenting of inventions involving elements of human origin” (N° 8, 25 September, 1996).

**Collection of biopsy and autopsy brain material for neuropathological studies**

**Italy** P1. Post-mortem human brain tissue is obtained from the UK Multiple Sclerosis Tissue Bank, in an anonymous form, together with information concerning the clinical disease course The use of this material has been approved by the Ethics Committee of the ISS. Given the limited amount of brain tissue obtained from the above bank, such tissue is usually utilized immediately for research purposes and therefore is stored only for a limited time period (12-24 months).

**Germany**: P2b. Gene expression studies and correlative studies on genotype and pathological phenotype will be performed on MS brain biopsy cases. These brain biopsy cases have been collected over the last years in the “MS Lesion Project”, an international collaborative effort supported by the National Multiple Sclerosis Society of the USA. Within this study, brain biopsy tissue is being collected from the patients included in the study. The storage and handling of the biopsy tissue is approved by the Ethics Committee of the Medical Faculty of the University of Göttingen. The amount of tissue obtained by brain biopsy is limited. The blocks are stored in the Department of Neuropathology for a period of about 15-20 years. Patients will be informed of the proposed storage arrangements.
All the patients have given their informed consent. The use of existing human tissue collections containing tissues from deceased patients has also been approved by Ethics Committee of the Medical Faculty of the University of Göttingen.

The approved procedure for collecting MS brain biopsy cases is as follows:

1. In the majority of cases, paraffin blocks are sent to the Department of Neuropathology of the University of Göttingen for an expert opinion (comparable to a Reference center) to confirm the diagnosis of an inflammatory demyelinating CNS disease consistent with multiple sclerosis.
2. If an MS-like inflammatory demyelinating process is diagnosed at the Department of Neuropathology (UK-GOE), we aim to include these patients in the present study. The ethics committee has therefore advised us to do so in the following manner:
3. We are not permitted to contact the patient directly. Therefore we contact the attending neurologists or physicians first to inform them about the study design (see attached P4_Letter 1).
4. We ask the attending neurologists/physicians to contact the patients and inform them about the study. The patients receive a “patient information sheet” (see attached P4_Letter 2) with detailed information about the study.
5. Patients can agree or refuse to be informed in more detail about the study by answering a form that is also sent to them. The patients also receive a postpaid envelope to send this form back to the Department of Neuropathology.
6. If the patients do not express interest, we are not permitted to contact the patients.
7. If the patients declare their interest in participating in the study, we contact them by phone and explain the study in detail. After this conversation, the patients give their informed consent to participate in the study (see attached P4_Letter 3) and sign an agreement for disclosure of confidential medical information (see attached P4_Letter 4).

Austria: P5. Gene expression studies and correlative studies on genotype and pathological phenotype will be performed on brain autopsy material, available in the archives of the Brain Research Center, Vienna. The work related to this project has already been approved by the Ethical Committee of the Medical University of Vienna (Ethik-Kommission der Medizinischen Universität Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH: Application Number: EK Nr: 535/2004; Project Title: Genetic determination of pathological heterogeneity of multiple sclerosis. Vote: Approval). According to the legal situation in Austria, autopsy material, when scientifically necessary, is stored indefinitely in the archives of the neuropathological institutions.

There is a worldwide debate about possible use of stored human tissues for research at universities and in the pharmaceutical industry. The laws related to this topic are at present unclear and vary from country to country. As a basis for the proposed project we have obtained a legal expert opinion from Prof Dr. C. Kopetzki, head of the Institute for Ethics and Law in Medicine, University of Vienna, confirming that the planned use of the tissue collection at the Brain Research Center, Vienna is in accordance with all relevant laws. This comprehensive opinion covers all applicable legislation in the areas of public health care, medicine, personal rights, data protection, genetics, civil law and criminal law, and university policies.

By Austrian legislation, existing human tissue collections containing tissues from patients that have died can be used for scientific investigation without specific informed consent as long as patient confidentiality is maintained and upon approval by the local ethical committee. This is in accordance with the emerging opinion in most European countries (for review see report from EU working group on Biobanks for Health “Optimizing the use of European Biobanks and Health Registries for Research Relevant to Public Health and Combating Disease”, Oslo 2003, and references therein)**.

** Additional references regarding human tissues in biomedical research: Beauchamp Tom L., Childress James F. Principles of Biomedical Ethics (fifth Edition). Oxford University Press Inc. 2001; MRC (Medical Research Council)UK; Ethics Series: Human Tissue and Biological Samples for use in Research (April 2001)
Commercialization of results obtained from research projects using human tissues is another important ethical issue. In order to develop highly specific new diagnostics and therapeutics, it is imperative to integrate human tissues in target evaluation and functional studies, the results of which may lead to financial gain for research and industry. In this context it is important to make clear that in our approach only scientific results but never the tissue itself or parts of the tissue will contribute to the value of a product. Every effort is made to reduce utilization of human tissue resources in order to preserve this valuable collection. By Austrian law, human tissues and material samples become the property of the physician in possession of the material. The tissues in the collection are stored indefinitely. Recent evidence demonstrates that archival paraffin embedded material allows studies on gene and protein expression by immunocytochemistry and in situ hybridization and is also suited for genotyping.

1) Research on human tissues

Collection of human blood samples for the generation of human CD8+ T cell lines

France: P6. Blood from multiple sclerosis patients (n=120) and patients with other neurological diseases (n=120) will be drawn for research purposes in the frame of a protocol approved on May 6th, 2004 by the Toulouse Ethics in Biomedical Research and Person Protection Committee in compliance with Huriet French law of December 20th, 1988. The protocol has been declared to the French Ministry of health and validated under the number 2004/0247. A detailed written information sheet is provided to the patients prior to their involvement in the study. A written consent of the patient is necessary before the research blood sample is drawn. All work involving the use of human biological samples will be performed following the French Bioethics law. It is clear that the research project on blood samples will be identified and known to the patients when they give their initial consent. The blood samples will be kept for the duration of the project and will thereafter be destroyed according to the French regulation. Long-term storage may be possible, if needed, but will require patients’ permission through a written informed consent for Biobanking. Converting, upon sample receipt, the patient’s name into a number code ensures anonymity. Only the number code is used in electronic files thereby following the French regulation.

Genetic studies

DNA samples have been donated by Swedish and Finnish MS patients who have given their informed consent of the samples being used for the identification of genes with an importance for the risk of multiple sclerosis (as described in a written patient information document). DNA samples have been obtained also from de-identified blood donors for whom information on ethnicity, sex and age has been preserved. Ethical approval has been obtained in the two centers (P8, P9) involved in research on genes determining susceptibility to MS. The basic requirements are that: 1) Informed consent is obtained from all patients in such a way that they clearly appreciate that the testing is for research purposes, that they will not personally benefit, and that refusal to consent will not in any way prejudice their health care; 2) the genetic data are stored anonymously on independent databases.

Sweden (P18): The Swedish gathering of DNA from persons with MS and controls is ethically controlled/overseen both by approval of a regional ethical committee and by a law on Biobanks which is in action since Jan 1st, 2004. The Neurogenetics Biobank is located in the Karolinska Hospital and is maintained according to the Swedish Act on Biobanks. To date, the ethical approval for genetic analyses in MS patients relates to three different projects: 1) The genetics of multiple sclerosis, approval under numbers 00-052 and 04-435; 2) The Stockholm prospective assessment of MS, approval under number 02-548; 3) The epidemiology of MS (EIMS), approval under number 04-252/1-4. The biobank law in Sweden establishes that every person has to agree on storing of samples for research and that the samples will be traceable if a person wants to withdraw from research and will be destroyed. Otherwise, biobanked material can be stored indefinitely in Sweden Two examples of the Patient Information sheets that will be used are attached (see P8_Patient_Info).

Sweden P3: Although initially involved only in designing markers and analysing genetic regions, there is the possibility that P3 will also be directly involved in work with patient material and will therefore apply for ethical permissions to use patient DNA.

Finland: P9. The legislation of medical research in Finland requires an informed consent for genetic studies from all study subjects. The consent can be given for a relatively broad phenotype. Each study must be reviewed by the ethical committee of the Helsinki University Central Hospital, which has NIH-certification. “Genetic study of Multiple Sclerosis” has been approved by the Ethics committee for Ophthalmology, Otorhinolaryngology, Neurology and Neurosurgery in the Hospital District of Helsinki and Uusimaa (Decision 46/2002, Dnro 192/E9/02). Also in this case, the patient tissue/DNA can be stored indefinitely.

Data storage and handling processes to ensure patient data protection and confidentiality
For all work involving databases of human information, electronic or otherwise, including genetic information, we will adhere to the law as laid down in the European directive 95/46 EEC on the protection of individuals with regard to the processing of personal data and on the free movement of such data. All patient material will be encoded to avoid identification of individual’s name or identity during material processing, so that genotypes that may be prejudicial to life or health insurance or employment cannot be linked to specific individuals. Confidential patient material including patient codes will be stored in locked cabinets accessible only to authorized and trained medical personnel directly involved in the study (P2b, P5, P8, P9).

In Sweden (P8), the databases are managed according on the Swedish Act on Personal Information, which has special rules for the storage of genetic information, and, in the case of P8, are under the supervision of the Karolinska Institute Hospital Officer of Personal Information.

In Finland (P9), the Office of the Data Protection Ombudsman, an independent authority operating in connection with the Ministry of Justice, must approve the general data collection. The Data Protection Ombudsman and the Office of the Data Protection Ombudsman provide guidance and advice on all issues related to the processing of personal data and control the observance of the law. No databases containing personal identifiers can be established, only study-specific local datasets. These must be destroyed when the project is closed.

Incidental clinical findings occurring while performing genetic studies or collecting brain bioptic tissues. These could occur if a certain gene or infection is found which could have dramatic consequences for the individual and, if not investigated in the study, would have gone unnoticed. In this case the patient will be informed by the treating neurologist. In case a genetic polymorphism should be discovered, which may have prognostic value for the disease course or, most importantly, therapeutic consequences, the patient will be also informed. As far as collection of bioptic brain samples in concerned, it is highly unlikely that an incidental finding may occur, as patients that have a diagnosis different from MS (e.g. tumor or infection) are not included in the study. In this case, the patient is directly informed about the diagnosis by the treating neurologist or neurosurgeon.

Animal experiments

Protection of animals

This project involves animal studies which are considered essential for the project development. The work will follow the national laws and the Council Directive 86/609/EEC from November 24th, 1986.
on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.

The consortium is aware of the opinion given to the European Commission by the “European Group on Ethics in Science and New Technologies” on “Ethical aspects of genetic modification of animals” (Number 7, 21st of May, 1996). The sufferance of all animal strains used in this project, both wild-type and genetically modified, will be kept at a minimum. In establishing an experimental protocol, the concept of the “3Rs” (Replacement, Reduction and Refinement) (Russell and Burch. The Principles of Human Experimental Technique; Methuen, London, UK, 1959; Poll et al., The ‘3Rs’ and the concept of alternatives in animal research: a questionnaire survey. Lab. Animal 33:47) will be followed as close as possible. Adherence to the above regulations will be subject to close and regular assessment and scrutiny by the Coordinator and the Steering Committee. Care will be taken to ensure that the studies are directly relevant to the project and that a minimum number of animals is used for the maximum return of information.

The major aims of the NeuroproMiSe project are the identification of the immunopathologic mechanisms underlying inflammatory neurodegeneration and of novel compounds for the treatment of severely disabling inflammatory neurodegenerative diseases. To achieve them, we cannot avoid to make use of established animal models of disease and to develop new models by transgenic technology, which mimic as close as possible the pathological situation in man, allow validation of the functional role of disease relevant genes, or permit the evaluation of the therapeutic potential of new protein/peptide drugs. The latter have proven to be invaluable tools with respect to mechanistic insights as well as for probing novel therapeutic concepts for a considerable number of human diseases. Moreover, in order to bring new reagents into the clinic and to obtain approval of new drugs for clinical use, it is presently mandatory to perform a preclinical testing on therapeutic activity, pharmacologic behaviour and toxicological aspects of the drug candidate in appropriate animal models.

Within this project, inbred mice and rats will be used. The most used animal model will be experimental autoimmune encephalomyelitis (EAE) in SJL and C57BL mice (P1, P3, P4, P6, P14, P17, P19), and in BN and DA (P2a, P8, P17, P18) rats. There are no valid alternatives to this animal model for examining complex immunopathological and neurodegenerative processes underlying neuroinflammatory diseases, which cannot be reproduced in an in vitro system, and for testing the effectiveness of potential neuroprotective and anti-inflammatory drugs already developed and to be developed by the consortium.

In addition, standard models of neurodegenerative disease, like sensory and motor nerve transection in rat strains (DA and PVG) (P8, P10), ischemia (P13, P14), and kainic acid-induced seizures (P14), will be employed for genetic, genomic, proteomic or therapeutic studies, which also cannot be replaced by in vitro systems due to the complexity of the combined inflammatory and neurodegenerative response that these models are aimed to reproduce. For the various disease models, standard operating procedures will be followed, as worked out by the participants and by animal guidelines of the NIH.

The generation of new mouse transgenic lines is justified as follows. The proposed research also involves the use of already existing and to be generated genetically manipulated animals, specifically transgenic, knockin, knockdown and knockout mice, which represent invaluable tools to validate the role of disease-relevant genes and test the therapeutic efficacy of compounds targeting human molecules. No conceivable alternative methodology exists that can surpass the analytical efficiency and speed of transgenic technology. Transgenic mouse models of disease allow research into pathological mechanisms, interactions between genetic and environmental factors, ways of approaching specific therapies and analysis of the long term safety of such intervention in humans.

Therefore, in practice, less rather than more animal experiments are required to reach the target of our research proposal since transgenic models of human disease more accurately represent the human disease and reduce the time and costs of primate and/or full scale clinical evaluation trials. All the transgenic mice that will be generated or used in the current project have already been or will be approved by appropriate Commissions in the respective countries. No abnormal phenotypes are expected from the transgenic mice that have been planned so far in this proposal, since the genes of interest are known to be non-essential for normal development, physiology and breeding. According to the Council Directive 90/219EEEC, the transgenic mice will be contained in their animal facility. To prevent any impact on animal biodiversity the animals will not be released in the wild.
Within the Subproject Identification, WPs I1, I2, I4, I5 and I6 aim to identify relevant genes involved in inflammatory and non-inflammatory neurodegeneration, and new targets for therapy. To verify the functional relevance of the most promising gene/protein candidates under pathological conditions, the generation of new transgenic, gene knockdown, knockin or knockout mice, as appropriate, will be essential and will rely on the expertise of several participant laboratories in conventional and conditional gene targeting technologies in mice.

Within the Subproject Mechanisms, WPs M1 and M2 aim at a refinement of neuroinflammatory disease models to provide better mechanistic insights into MS immunopathology (in particular into CD8 and CD4 T-cell-mediated CNS tissue damage) and at creating humanized transgenic mice that can be utilized for testing therapeutic agents. A requirement for new transgenic lines also stems from the need to preclinically test reagents that exert strict species specificity, such as antibodies or proteins directed towards human targets. For this, humanized transgenic mice will be created by knock-in strategy (expressing human TNFRs instead of murine; WP V3 in subproject Validation) that allow appropriate analyses of the in vivo efficacy of potential therapeutics targeting TNF receptor subtypes. As murine TNF binds to both human TNF receptors, but not vice versa, these animals should have a completely normal physiology and, in addition, be able to respond to human specific reagents. For this class of protein therapeutics, there is no alternative to obtain an in vivo functional assessment at a preclinical level.

The use and generation of transgenic mice will be performed in 7 laboratories (Germany, P7 and P19; Sweden P3; UK P4; France P6; Finland P9; The Netherlands P13; Greece P14), all recognized as expert laboratories with a long record in the field of transgene technologies, holding permissions to create and breed transgenic lines in accordance with the national and EU regulations on animal welfare as well as genetic engineering and safety (like the German Act on Genetic Engineering (Gentechnikgesetz) 1993, and guidelines set down in the Commission de Génie Génétique in France, and EC Council Directive 98/44, L213/13 July 30, 2000). The generation of transgenic mice in these laboratories follows standard procedures. Genetically modified mice are housed in authorized, fully equipped, pathogen-free animal facilities and handled by experienced animal care takers and personnel. As a regular procedure, toxicity tests of animals are conducted and mice are considered as pathogen free. Specific-pathogen-free (SPF) areas are only accessible for particularly educated personnel. For transportation, mice will be transferred to certified transport-boxes supplemented with food pellets and water repositories guarantying survival for up to a week.

Animal experimentation and authorizations in the participating countries

Italy: P1. All experiments will be conducted in accordance to Council Directive 86/609/EEC and decree 116/92. All experimental procedures related to the establishment of mouse EAE models and brain cell cultures have been approved by the Italian Ministry of Health. Authorization for injecting lentiviral vectors in the cisterna magna of mice will be requested, upon approval of this project.

Germany: P2a, P7, P19. All experiments will be conducted in accordance to Council Directive 86/609/EEC and the German ‘Tierversuchsgesetz - TVG (1988). All experimental procedures related to this project are already approved by the responsible local authorities in Germany (Regierungspräsidium Braunschweig, Kuhstr. 18, 38022 Braunschweig, Germany). For P3, the approval is registered under the reference number 509.42502/01-14.04. For the generation of transgenic mice and the conductance of animal trials, authorization from the 'Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES) has been granted to P9.

Sweden: P3, P8, P17. Animal experimentation is regulated and authorized by the Swedish animal welfare agency. For use of laboratory animals, a permit is released for keeping, breeding and performing animal experiments. Seven local ethical committees, located in the local courts, handle permission for animal experiments (permission #168-2005 has been delivered to P17 for establishment of EAE in rats) for. National guidelines for the 3 Rs have been developed by the Swedish agricultural ministry. Specifically for EAE, rats or mice should be euthanized if decreasing more than 15% in weight, developing severe paresis or severe bladder problems, or otherwise showing signs of pain or suffering. A veterinarian oversees this

P3: The local animal ethical committee of the University of Lund has already approved all planned experiments for the next three years, which includes EAE models, identification of mice and rats for genotyping, and transgenic experiments including embryo transfer and caesarean sections. Authorizations are as follows: Project title: Identification for genotyping of rodents in breeding and
experiments; approved 03/05/2004, M75-04; 2) Project title: Studies of animal models for multiple sclerosis (MS); approved 03/05/2004, M69-04; 3) Project title: Use of animal models for rheumatoid arthritis (RA) with the purpose to identify and study genes involved in the disease; approved 03/05/2004, M70-04; 4) Project title: Embryotransfer and caesarean section of rodents; approved 28/06/2004, M125-04.

P8: All the experimental procedures related to this project have been approved by the local animal ethical committee, with permission numbers N 266/04 and N 375/03.

P17: For establishment of EAE models in mice and rats, permission has been obtained from the local ethical committee in Malmö and is valid until june-2007 (#M69-04).

Finland: P9. All animal experiments must be accepted by a national board of animal welfare. This board monitors the experimental details as well as principles of the 3R’s. Authorizations for knock-out mice used in this project are as follows: for Dap12, R2004-06/STU385A-2004; for Trem: R2003-23/STU1164A-2003.

UK: P4, P10, P11, P18. All research involving animals in the UK is strictly regulated under the “Animals (Scientific Procedures) Act 1986” and in accordance with Council Directive 86/609/EEC. Applications must first pass an internal review committee, and then a government inspector before the application might be approved and a licence granted by the UK Home Office. The Home Office will only permit work to be done if: i) it has been approved by the Local Ethical Review Committee; ii) full attention and consideration has been given to the 3Rs; and iii) a proper cost benefit for animal welfare and possible outcomes has been considered. All UK animal research laboratories are subject to Government inspection without warning. Each person who undertakes work under the Act holds a personal licence issued by the UK Home Office. The personal licenses are only approved after the attendance of a Home Office approved training course and are valid for 5 years. P4, P10, P11 and P18 hold all the project licenses (PPL30/1890, PPL 50/5972, PPL70/5649, and PPL70/5625, respectively) covering the animal experiments related to this project.

France: P6. Animal welfare requirements will be respected according to the Amsterdam Protocol, to the Council Directive 86/609/EEC, and to the current French legislation, regulations and guidelines (decree 2001-486). P6 holds the licence number 006462 for performing experiments on vertebrate animals, delivered by the French Ministry of Agriculture in compliance with the decree 87-848 and interministry decree of April 19th, 1988. The agreement for this laboratory animal care facility was formally attributed following the interministry decree of April 19th, 1988 to the animal facility of IFR30 on April 22nd, 2003 with the number B-31-555-8. The transgenic and non-transgenic mice studied will be bred in a fully authorized animal house, the IFR30 animal facility. Although this is not a mandatory procedure in France, all animal protocols implemented in the current project have been submitted to the Midi-Pyrénées region committee on ethics for animal research and have been approved on May 24th, 2004. This committee has approved the method of mouse euthanasia employed (cervical dislocation) following decree 2001-464 of May 29th, 2001 modifying decree 87-848 of October 19th, 1987. The transgenic mice (GMO) that will be generated or used in the current project have received the agreement number 3986 from the genetics commission of the French Ministry of Education and Research on October 23rd, 2003, in accordance with the French law 92-654, decrees 93-773, 93-774 and decree 98-18.

The Netherlands: P13. Animal welfare requirements will conform to the Council Directive 86/609/EEC. The necessary authorizations for generating and using transgenic animals and animal models pertinent to this project have been issued by the Ministrie van Landbouw, Natuur en Voeiselkwaliteit, Commissie Biotechnologie bij Dieren (VVA/BD03.90 (G03) and by the local animal experiment committee (DEC D4058A, DEC D4048A and DEC 3056), as well by the Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer, Centrum voor Stoffen en Risicobeoordeling, Bureau GGO (GGO03-167).

Greece: P14. All experiments will be conducted in accordance to Greek law 2015 /27.2.1992 and Council of Europe Directive 86/609/EEC. For the use of laboratory animals at the Experimental Animal Unit of the Hellenic Pasteur Institute, permits have been issued for breeding (978/1 20.4.1993), distribution (978/2 20.4.1993) and experimentation (501 5.4.1993) by the Greek State, Nomarchia Athinon, Diethnhsis Ktiniatrikis, and are maintained through routine inspections by state officials. The same official body has already approved all planned experiments for 2003-2004 in Animal License entitled: Production and analysis of transgenic and gene targeted mice; approved 01/07/2004, K/3501 (responsible scientist Dr L. Probert), and this License will be renewed annually.
The Greek research team has held animal licenses for work involving the production and analysis of transgenic and gene targeted mice continuously since 1994. The animals that will be used for this project are bred and maintained in the SPF Transgenic Unit of the Experimental Animal Unit of the Hellenic Pasteur Institute, Athens, which has been purpose built and fully equipped, and is staffed for the generation and housing of transgenic and knockout mice. Institute guidelines for the 3R’s are in place. Specifically for EAE, rats or mice should be euthanized if their clinical score exceeds 3.5 (acute weight loss with hind-limb paralysis, bladder incontinence). For kainic acid-induced seizures, seizures will be closely monitored using universal clinical scoring criteria and mice should be euthanized if their clinical score exceeds 4 (1, arrest of motion; 2, myoclonic jerks of head & neck, twitching; 3, unilateral clonic activity; 4, bilateral forelimb tonic & clonic activity; 5, generalized tonic-clonic activity, loss of posture, including death from convulsions). Following permanent focal occlusion of the left middle cerebral artery (pMCAO) (ischemia model), the head incisions are closed and the mice are allowed to recover from anaesthesia in 30°C incubator (dark, quiet) and subsequently returned to their cage in the experimental unit with free access to food and water and supervision and if necessary nursing by the in-house veterinarian. Topical application of lidocaine (Xylocaine) or Macaine jelly at the site of the incision prior to recovery from anaesthesia will ensure the absence of discomfort from the surgery. Animals will undergo a single control surgery to achieve a sham operation for MCAO that will involve opening and closing of skin and bone on one side of head. Mice will be allowed to recover as described above. Following ischemia surgery, mice usually recover well and show good health. However, if any mice show signs of distress or ill-being they will be euthanized. Animals will be allowed to survive until sacrifice at selected time points ranging between 3 hours and 14 days after surgery. Animals will be sacrificed and tissue samples will be collected as described for EAE above. In all cases, euthanasia is humanely achieved by regulated carbon dioxide gas inhalation or sevoflurane inhalation.

Fulfilling the 3 Rs
Specifically designed animal departments with trained technicians and veterinarians are present in each of the participating institutions, with the goal to fulfil the 3R’s.

Reduction: All efforts will be made to reduce the numbers of mice and rats used in this project. This will be achieved by applying the most efficient breeding and experimental strategies. The minimum number of mice or rats compatible with obtaining sufficient tissue for microarray and proteomic approaches will be used. At the end of each experiment carried out in animal disease models, organs including brains, livers, lymph nodes and spleens, will be stored and used for multiple experimental purposes. Mouse/rat tissues used for microarray and proteomic approaches will be properly stored and additionally used for histological examinations and validation analysis. Experiments will be carefully planned to maximise the information obtained per animal and thus to limit the subsequent use of additional animals. The minimum number of animals will be used consistent with obtaining statistically significant findings. Experiments aimed to analyze the molecular mechanisms of new therapeutics will evaluate disease susceptibility and treatment biomarkers that can be used to follow disease progression and treatment efficacy. Such molecular diagnosis markers will enable an early prognosis of disease and treatment efficacy that will reduce the number of animals that experience disease chronicity or even disease initiation.

Refinement: Inbred rats and mice are the animals of choice for the proposed research as much background information is available for these species, and they can be ethically used in the limited numbers necessary. The inbred and transgenic animals that have been chosen for in vivo experiments represent well-characterized models for neurodegeneration and the corresponding human diseases under study (in particular multiple sclerosis). Their use allows a strict control of genetic background and accurate timing of lesion development, so that the number of animals required to achieve statistically significant results is minimized. For most experiments, the C57BL/6 mouse strain is chosen because this represents a genetic background that is compatible with most experimental CNS disease models, and is the background of choice for the majority of transgenic and knockout animals. Refinement is achieved by using numerous approaches. Animals will be housed in a physiologically controlled environment and will have free access to food and water. When possible, environmental enrichment will be introduced in order to avoid boredom and animals will be routinely checked for the recurrence of behavioural stereotypes. In the specific pathogen-free transgenic units, all animals are
housed and maintained under excellent conditions of cleanliness and hygiene by full-time trained staff and in-house veterinarians, as well as responsible scientific staff that is highly trained in recognizing changes in mouse/rat behaviour and phenotype including signs of pain, distress or ill-being. For EAE experiments, the mice will be monitored daily for signs of neurological disease, according to the clinical scoring system, which allows good monitoring of distressed animals. If mice show signs of malaise, e.g. hunched posture, staring coat, immobility, or if the clinical score reaches 3.5-4, they will be sacrificed. Transgenic animals exhibiting any unexpected harmful phenotype will be humanely killed, or in the case of individual animals of particular scientific interest, advice will promptly be sought from the local ethics committee. Care will be taken that the animals are not kept in experiments longer than necessary, and no animal will be used in more than one experimental procedure. All experimental procedures in this project will be carried out with the maximum effort to avoid or minimize discomfort, distress and pain to the animals, and procedures that will necessarily cause more than momentary or slight pain or distress to the animals will be performed with appropriate analgesics or anaesthetics in consultation with the attending veterinarian. All surgical procedures will be performed under general anesthesia, using controlled systems. Recovery of the animals will occur in a specially arranged environment on warm bedding and under supervision of technicians.

Replacement: To minimize the use of experimental animals, major efforts will be made to use primary cultures of immune cells (P3, P4, P17), neurons and glial cells (P1, P13, P14, P19), and in vitro viral transduction of neuronal/glial cells (P14, P19), both for investigating immunopathological and neurodegenerative mechanisms and for testing the efficacy and mechanism of action of neuroprotective compounds. We shall always first evaluate the possibility to validate the role of candidate molecules/pathways emerging from genomic/proteomic studies using in vitro systems, before proceeding to their analysis in established animal models or to the generation of new transgenic models. Using RNAi strategies in vitro, gene functions will be initially verified and characterized in cell culture systems. The tissues and cell material required for the in vitro experiments will be prepared from embryonic, newborn or adult mice, according to established procedures upon sacrifice of animals. To avoid to use cells from multiple mice, T cell clones will be generated that can be expanded in vitro. It will not be possible to substitute primary cultures of neural cells with neuronal/glial cell lines since the latter generally carry mutations in the survival/proliferation/apoptosis pathways that will be studied in this project or likely be affected by the therapeutics under investigation.

Genetically modified organisms

Within this project, retroviral vectors, particularly lentiviral vectors of the third generation, will be used to genetically modify cultured cells (P14, P19), induce ectopic expression of cytokines/chemokines in the CNS in vivo (P1), and generate transgenic mice (P3, P19). Lentiviral vectors have been shown to be highly efficient in transduction of all types of target cells including neural cells, stem cells and early embryonic cells. All the participants involved in research with lentiviral vectors hold permission to manipulate OGM. The lentiviral vectors that will be generated or used in the current project have already been or will be approved by appropriate Commissions and will be manipulated in authorized laboratories of safety level S1 and S2.

Another major scientific goal of the project is the development and in vivo evaluation of recombinant protein therapeutics selectively targeting TNFR1 and TNFR2 (P12, P13) and neurotrophic pathways (TAT fusion proteins) (P2a). To this purpose, new reagents have to be developed and produced by standard genetic engineering techniques involving genetically modified organisms for protein expression. This concerns in particular various cytokine derivatives of TNF and fusion proteins on the basis of TNF. The construction and generation of these novel, recombinant molecules comprises expression in prokaryotic (E.coli) and eukaryotic (Yeast, SF9 insect cells, mammalian cell lines) organisms according to established, standard protocols. This work has been evaluated and classified as safety level 1 according to German Legislation on work with genetically modified organisms. It will
be carried out in the laboratories of P2a and P12 (Germany), in registered laboratories of safety level S1 and S2 (Zugelassene gentechnische Anlagen nach Gentechnikgesetz, S1 Reg No. 57-13/8817.40-020/UNI.S.06.11; S2 Reg. No. 76-14/8817.02/UNI.S.06.09).

The Steering Committee will take all measures to ensure that appropriate environmental safety provisions are fulfilled in the course of the project by all contractors, particularly those related to deliberate release into the environment of genetically modified organisms. In addition, the coordinator and the steering committee shall take all measures to ensure that all participants, when dealing with potentially hazardous biological material, will observe strict safety procedures, in compliance with national and EU regulations on biosafety.

Ethical issues will be dealt with by the management group (the coordinator and subproject leaders) of the consortium and a workshop on ethical issues will be organized at the beginning of the project, as part of the training activities. During this initial workshop, and with the assistance of an expert in the field, legal and regulatory issues in the Member States and within the EU will be discussed, with the purpose to develop an internal code of conduct for procedures adopted in the different tissue banks, which can be applicable to all countries involved in the project. As stated above, the research activities carried out by the partners of NeuroproMiSe already strictly fulfil both national and EU legislation criteria. Nevertheless, the Coordinator and the Steering Committee will ensure that all research regulated by national or EU guidelines is initiated only after obtaining the relevant authorizations. In particular, local or national ethics committee approvals and appropriate licences will have to be submitted to the SC in all cases. Moreover, the contracts to be signed between coordinator and partner laboratories will enforce that ethical issues will be addressed properly by each participant according to EU legislation. The management board will monitor justification of newly planned animal experiments in partner’s laboratories and, upon acceptance, will forward these to the EU commission. No partner will receive any financial support before all relevant approvals have been forwarded to the Coordinator and all procedures for ensuring patient confidentiality have been clearly stated. In case of controversial issues or new ethical regulations arising, the management board will seek advice from independent external authorities. The management of the consortium will take all measures to transmit new regulation or decisions derived from these authorities into effect in the relevant partner laboratories. The Commission will be informed specifically by the Coordinator of all authorizations obtained by the partners.
APPENDIX A - DESCRIPTION OF THE CONSORTIUM

A.1 PARTICIPANTS AND CONSORTIUM

The NeuroproMiSe consortium is composed of 19 legal entities of which 15 are academic and public research institutions and 4 are biotechnology companies (of which 3 are SMEs), with a total of 20 actively participating research groups from 9 EU Member States. Two independent research groups (P2a and P2b) are from UKG-GOE (Contractor #2). Various partners have successfully participated in former EU projects and some have acted as coordinators (P2a, P3, P5, P8, P9, P10, P14, and P16). The partners are all European leading experts in genetics, neurology, neuropathology, basic neuroscience and neuroimmunology and will provide expertise, insight, personnel, existing infrastructure and resources to ensure implementation of the proposed activities. The merging of these activities in a common, objective-driven project will not only allow a better usage of resources and knowledge but will also permit rapid validation of candidate genes and evaluation of the efficacy of potential therapeutics with anti-inflammatory and neuroprotective activity in a wide variety of model systems and in different combinations. Most participating laboratories will contribute to more than one task of the proposal, allowing cross-disciplinary research and training as well as scientific and technological integration.

With respect to the realization of the project aims, the consortium presents the following major advantages:

1) The consortium comprises excellent research groups in the field of neuroinflammation/neurodegeneration and related experimental and human diseases. Over the last 20 years, the participants have provided key contributions in the identification of genes relevant for human and experimental neuroinflammatory diseases, immunopathological and neurodegenerative processes involved in MS, biology and pathology of neurons and glial cells, and generation of lead products of this project.

2) The consortium has complementary expertise. The participants are internationally recognized as leading groups in human and animal genetics (P3, P4, P8, P9), neuropathology of MS and EAE (P2b, P5, P10), basic mechanisms of CNS inflammation (P1, p3, p8, P10, P14, P19) and axonal/neuronal damage (P2a, P10, P11), T-cell autoimmunity in MS (P4, P6), myelin biology and demyelinating diseases (P7), glial cell biology (P1, P19), and TNF biology (P12, P13, P14).

3) The consortium includes 4 biotechnology companies, which provide proprietary techniques and competence in product development relevant to the aims of the project. Their specific competences are in the development of protein based recombinant drugs with emphasis on anti-TNFR monoclonal and polyclonal antibodies (HBT, P16) and TNF-R selective agonists (P20), in the identification of molecular targets in MS and chemical approaches to the development of anti-inflammatory drugs (ARX, P17), and in the development of neurotransmitter receptor and ion channel blockers (ELL, P18).

4) Cooperation between members of the consortium is extensive and a number of bi- and multilateral collaborations already exist, some since several years, as evident from joint publications (see below).

5) The consortium utilizes state of the art technologies in genetics (high throughput SNP genotyping) and functional genomics (microarrays, proteomics, gene targeting and expression technologies in mice) which will be utilized to identify and validate disease-relevant genes and their products (P3, P7, P8, P9, P10, P14, P17, P19), and to generate new animal models (including humanized transgenics) to be used for analysis of pathogenic mechanisms of neuroinflammation and testing of therapeutic compounds (P4, P6, P13).

6) The consortium participants hold all the technologies and patents for genetic engineering and production of recombinant proteins and bifunctional molecules to target specific neural cell populations (P2a, P12, P14, P16) for therapeutic purposes.

7) The consortium has a unique animal platform for genetic studies, which is established at ULUND (P3) and KI (P8). This platform will be used to map and characterize genes relevant for MS and
neurodegenerative disease models and the results obtained in the experimental models will guide the genetic studies in MS patients and control populations which will be performed by P8 and P9.

8) The consortium has unique access to well characterized, homogeneous cohorts of MS patients and matched controls for genetic studies (P8, P9) and to the world's largest collection of well characterized biopsy and autopsy material from MS and other human inflammatory CNS diseases (P2b, P5).

9) Most consortium participants have profound experience with different animal models of acute and chronic CNS inflammation, including mouse and rat EAE (P1, P2a, P3, P4, P6, P8, P11, P14), brain ischemia (P13, P14), traumatic brain injury (P8, P10), and non inflammatory neurodegeneration (P7), as well as in vitro systems of neurons and glial cells (P1, P13, P19), which will be utilized for gene validation, investigation of pathogenic mechanisms and testing of therapeutic compounds.

10) The consortium has organized horizontal activities, which will provide adequate scientific and technological support to all consortium partners for those activities involving neuropathological characterization (P2b, P5), high throughput genomic and proteomic screens of relevant animal and human tissue samples (P3, P9, P10), integration and evaluation of the data obtained in expression studies (P14, P17).

11) The consortium represents a unique ‘teaching resource’ for both internal and external young scientists, and partners of the consortium are already engaged in teaching activities at the European level.

12) The consortium has already agreed on the definition of a Consortium Agreement and of an Advisory Board, which will monitor the project. By June 22nd, 2005 all partners and their legal offices have viewed and approved the final version of the Consortium Agreement.

The specific competence and contribution of the individual partners to the scientific subprojects of the the NeuroproMiSe project is summarized in the following table:

<table>
<thead>
<tr>
<th>Participant</th>
<th>No</th>
<th>Competence</th>
<th>Role in the Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloisi/ISS</td>
<td>P1</td>
<td>Glial cell biology, chemokines, immunopathology of EAE/MS</td>
<td>EAE models with CNS ectopic lymphoid tissue; lentivirus-mediated CNS expression of cytokines/chemokines regulating B cell responses; laser microdissection; confocal microscopy; analysis of ectopic lymphoid tissue in the MS brain; glial cell (oligodendrocytes, astrocytes, microglia) cultures for evaluation of neuroprotective compounds; glial cell functional assays</td>
</tr>
<tr>
<td>Bähr/UKG-GOE</td>
<td>P2a</td>
<td>Neurodegeneration, apoptosis, intracellular signalling pathways</td>
<td>Animal model of MS with optic nerve degeneration; analysis of apoptotic pathways in neurons; development of anti-apoptotic therapies and HIV-TAT-fusion proteins; functional evaluation (evoked potentials) and MRI assessment of therapeutic strategies</td>
</tr>
<tr>
<td>Brück/UKG-GOE</td>
<td>P2b</td>
<td>Neuropathology of MS/EAE; MS brain biopsies</td>
<td>Validation of gene polymorphisms in MS brain tissue; neuropathological analysis of animal models; organization of the Neuropathology Reference Center</td>
</tr>
<tr>
<td>Holmdahl/ULUND</td>
<td>P3</td>
<td>Genetic analysis of animal models of MS; positional cloning of disease relevant genes; functional analysis of candidate genes</td>
<td>Identification of disease-relevant genes in congenic mouse strains and validation of their functional role; EAE models; functional genomics; bioinformatics; mutated and transgenic mice; ex-vivo immunological studies</td>
</tr>
<tr>
<td>Fugger/MRC</td>
<td>P4</td>
<td>Genetics of MS; MHC genes; humanized animal models</td>
<td>Validation of the human polymorphisms (e.g. MHC, CIITA) in animal models; role of MHC class I and class II molecules in CNS autoimmunity; generation of humanized animal models expressing MS-relevant MHC class I and class II molecules and T-cell receptors</td>
</tr>
<tr>
<td>Lassmann/MUW</td>
<td>P5</td>
<td>Neuropathology of MS, EAE and other neurodegenerative diseases</td>
<td>Validation of candidate genes in pathological brain samples; neuropathological analysis of animal models; organization of the Neuropathology Reference Center</td>
</tr>
<tr>
<td>Liblau/INSERM</td>
<td>P6</td>
<td>Clinical and experimental neuroimmunology; humanized animal models</td>
<td>Development of animal models (including humanized animal models) to study the pathogenic role of encephalitogenic CD8+ T cells and evaluation of...</td>
</tr>
<tr>
<td>Project/Institution</td>
<td>Person</td>
<td>Work</td>
<td>Description</td>
</tr>
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</tr>
<tr>
<td>Nave/MPG</td>
<td>P7</td>
<td>Myelin and oligodendrocyte biology, demyelinating diseases</td>
<td>Myelin mutant mice; laser microdissection of fluorescently labeled neurons and glial cells; cDNA microarrays; transgenic mice</td>
</tr>
<tr>
<td>Olsson/KI</td>
<td>P8</td>
<td>Genetic analysis of MS and animal models of MS and neurodegeneration; clinical and experimental neuroimmunology</td>
<td>Identification of disease-relevant genes in congenic rat strains and validation of their functional role; rat models of EAE and neurodegeneration; functional genomics; identification of gene polymorphisms in Swedish MS patients; high throughput SNP analysis</td>
</tr>
<tr>
<td>Peltonen/NPHI</td>
<td>P9</td>
<td>Genetics of human diseases; molecular pathogenesis of mutated genes; animal models</td>
<td>Identification of gene polymorphisms in Finnish MS patients; high throughput SNP analysis; DAP12 mutant mice; cDNA microarray analysis; bioinformatics</td>
</tr>
<tr>
<td>Perry/USOU</td>
<td>10</td>
<td>Mechanisms of CNS inflammation and neurodegeneration in human and experimental diseases; innate immunity</td>
<td>Animal model of Wallerian degeneration; influence of systemic infection on neurodegeneration; microglia reactivity; inflammatory mediators in the CNS; proteomics; bioinformatics</td>
</tr>
<tr>
<td>Smith/KCL</td>
<td>P11</td>
<td>Axonal pathology in demyelinating and neurodegenerative CNS diseases; NO-mediated neurotoxicity</td>
<td>Testing of sodium channel and sodium/calcium exchanger blockers in <em>in vitro</em> and <em>semi-in vivo</em> spinal cord preparations; electrophysiological and morphometric studies; focal demyelinated lesions</td>
</tr>
<tr>
<td>Pfizenmaier/USTUTT</td>
<td>P12</td>
<td>TNF signaling, apoptosis, recombinant proteins, targeting</td>
<td>Human TNF receptor selective agonists and antagonists</td>
</tr>
<tr>
<td>Eisel/USGR</td>
<td>P13</td>
<td>TNF signaling, neuroprotective signalling pathways, transgenic and mutant mouse approaches</td>
<td>Development of humanized mouse models for TNFR subtype activation; models of brain ischemia; cultured neurons</td>
</tr>
<tr>
<td>Probert/HPI</td>
<td>P14</td>
<td>Cytokine signaling in neuroinflammation; transgenic mouse models of CNS inflammation</td>
<td>Validation of TNFR1 signaling compounds in animal models of acute and chronic neurodegeneration; integration of data sets from cDNA microarray analyses of different disease models and <em>in silico</em> simulation of the CNS inflammatory response and disease-related pathways.</td>
</tr>
<tr>
<td>Buurman/HBT</td>
<td>P16</td>
<td>Biotechnology of recombinant proteins; monoclonal and polyclonal Abs</td>
<td>Production of antagonistic huTNFR1 specific Fab and scFv (H398 derivatives), murine TNFR1 and TNFR2 specific antagonistic/agonistic mAbs to test in <em>in vitro</em> and <em>in vivo</em> disease models</td>
</tr>
<tr>
<td>Olofsson/ARX</td>
<td>P17</td>
<td>Molecular genetics; target validation in animal models; pharmacotoxicology</td>
<td>Validation of Ncf-1 in EAE development; development of Ncf1 targeting compounds with therapeutic efficacy in animal models of MS; bioinformatic integration: genotype-phenotype data base integrating the results of genomic and proteomic studies obtained in animal models</td>
</tr>
<tr>
<td>Patel/ELL</td>
<td>P18</td>
<td>Small molecule drugs</td>
<td>Development and testing of glutamate antagonists and calcium channel blockers for neuroprotective activity <em>in vitro</em> and in animal models</td>
</tr>
<tr>
<td>Neumann/UNI-BONN</td>
<td>P19</td>
<td>CNS innate immunity, mechanisms of axonal and neuronal damage</td>
<td>Studies in TREM-2/DAP12 mutant mice and TREM-2 knock-down mice; <em>in vitro</em> models for the study of microglia-axonal interactions and evaluation of neuroprotective compounds; axonal transport; intracellular signaling pathways; lentiviral vectors for expression of GFP-tagged synaptic and mitochondrial genes; lentivirus-mediated RNA interference of microglia pro-inflammatory genes; time lapse microscopy and confocal/2-photon laser scanning microscopy</td>
</tr>
</tbody>
</table>
Role of the Consortium participants in the administrative subprojects

Subproject Training: Six participants from 5 EU countries will contribute to this subproject. The coordinator of the Subproject Training (P14) is group leader at the Hellenic Pasteur Institute and supervisor of several training programmes of HPI. P2a is chair of the Neurology Department of UK-GOE, which is associated with an International Graduate School for MD-PhD in Neurosciences and participates in an EU-funded research training program for predoctoral neuroscientists. P5, P6, P8 and P14 are members of the scientific committee and course organizers/instructors for the European School of Neuroimmunology (ESNI). P3, P6, P19 and P5, who act as coordinators or co-coordinators of the Subprojects Identification, Mechanisms, Validation and Horizontal integration, respectively, will ensure that partners involved in the respective subprojects participate actively in training activities and will take care, under the coordination of P14, that Marie Curie fellowships are applied to spread the training in the scientific community outside the project. The coordinator of the Subproject Training will also ensure complementary training in the field of knowledge management, ethical and society issues.

Subproject Management: Eight partners from 7 EU countries participate to this subproject. ISS (P1) is the coordinator and UNI-BONN (P19) is the Co-coordinator of the Suproject Management, in close cooperation with the coordinators of all the other Subprojects and in accord with the decisions taken by the General Assembly. The coordinators and co-coordinators together with one business management representative (Dr. Giovanni Cozzone, ISS) form the Steering Committee (SC), which will be the executive body of the Project. The SC, through the Coordinator and the Co-coordinator, will oversee both the scientific and the administrative structure of NeuroproMiSe, which are the executive arms of the project. The SC will implement the strategy of the project and execute key decisions taken by the General Assembly. The major tasks of the Subproject Management are to monitor the progress of the project and to ensure that it maintains its objectives and relevance within the research topic LSH-2004-2.1.3-2, to validate the scientific and financial reports and to review the financial status of the project. The coordinator and co-coordinator of each subproject will coordinate the activity of the corresponding subproject to ensure that the scientific, technological, and training objectives will proceed as scheduled.

NeuroproMiSe participant profiles

P1/ISS
The institution: The Istituto Superiore di Sanità is the technical scientific body of the Italian National Institute of Health. It has scientific, organizational, administrative and accounting independence and is subjected to monitoring by the Ministry of Health. The Department of Cell Biology and Neurosciences consists of 11 independent units, with a total of about 250 scientific, technical and support staff and has been recently established with the aim of promoting basic and applied research as well as surveillance activities in several human pathologies, including chronic neurodegenerative and neuroinflammatory diseases. The Institute is fully equipped with all the facilities that are necessary for molecular biology, tissue culture, microscopy, flow cytometry, manipulation of lentiviral vectors and animal breeding.
The principal investigator: Francesca Aloisi, PhD, is Coordinator of the NeuroproMiSe project, Coordinator of the Subproject ‘Mechanisms’ and leader of WP M3. She is group leader in the Department of Cell Biology and Neurosciences and has been working in the fields of Neurobiology and Neuroimmunology since 20 years, focusing on glial cell biology and immunocompetence, basic mechanisms of neuroimmune interactions and immunopathogenesis of EAE and MS. She is PI and Co-PI of National Projects on multiple sclerosis and other chronic neurodegenerative diseases. She is President of the Italian Association of Neuroimmunology, Member of the Advisory Board of the International Society of Neuroimmunology and Member of the Editorial Board of the Journal of Neuroimmunology and Glia. She will spend 70% of her time on this project.
Research group members involved in the IP and their experience:
Elena Ambrosini, Research scientist (8 years): Molecular/cellular biology, protein expression/purification, transfection techniques.
Maurizio Federico, Senior Research Scientist (11 years): Specialist of HIV replication research; Retro- and lentiviral vector-based gene therapy.
Barbara Serafini, Research Scientist (12 years), Immunohistochemical techniques, confocal microscopy, laser microdissection, animal models.
Sandra Columba-Cabezas, Technician (12 years): Molecular biology, animal models.
Cristina Agresti, Research scientist (15 years): Brain cell cultures, organotypic cultures, biological assays (proliferation, migration, survival, cytotoxicity), immunocytochemistry.

Five relevant publications:

P2a/P2bUK-GOE
Partner UKG-GOE comprises two independent groups, each headed by a Principal Investigator (P2a, P2b, respectively) and belonging to a different Institute/Department within UKG-GOE. P2a, Chair of the Department of Neurology, is the person in charge of UKG-GOE. The University of Göttingen is known for its scientific strength in Neuroscience.

P2a/UK-GOE
The institution: The Department of Neurology of UKG-GOE has a long-standing tradition in MS research. Prof. Bauer, a former head of the department was one of the first to introduce neurochemical methods into the MS field. Since then, the department has had a focus on clinical and experimental neuroimmunology and has acquired one of the largest regional databases on MS patients. Since Mathias Bähr was appointed clinical department chairman in 2001, the department has been newly equipped with the infrastructure for basic research. In 2002, the first Institute for Multiple Sclerosis (IMSF) was established. The Department of Neurology is also associated with an international Master/PhD Max-Planck Research School in Neurosciences and Molecular Biology and participates in 2 EU-funded research training programs for predoctoral neuroscientists (NSR and Neurest). The Journal of Neuroscience, Neurobiology of Disease.

Research group members:
Ricarda Diem, MD, is the senior scientist and leader of this research group. Helmut-Bauer-Award 2004 for MS research. Since 6 years actively working in the field of neurodegeneration, protection and neuroimmunology. Profound experience in animal models of multiple sclerosis, in vivo electrophysiology, patch clamp techniques, mechanisms of neuronal degeneration and development of neuroprotective treatment strategies. Muriel Sättler, MD, postdoctoral fellow (> 4 yrs): axonal and neuronal injury in animal models of MS, expert on neurotrophic-dependent signal transduction, in vivo electrophysiology, molecular biology. Katharina Maier, MD, postdoctoral fellow (> 3 yrs): in vivo models, neuroprotective treatment studies, current focus of work on protective effects of CNTF in EAE, immune histology. Iris Demmer, MD, postdoctoral fellow (> 2yrs): in vivo electrophysiology, MRI visualisation of optic nerve inflammation in rodents. Ina Boger, is a trained technician (> 3 yrs): expert in the field of immune histology, microscopy and protein analysis.

Five relevant publications:
The Department of Neuropathology of UKG-GOE has a long-standing tradition in basic and applied clinical research of neurodegenerative and neuroinflammatory disorders. In 2002, Wolfgang Brück was appointed department head and since then the department has been restructured and newly equipped with the infrastructure for experimental and in vitro research. The main scientific focus of the department are clinical, pathological and experimental approaches to clarify the pathogenesis of inflammatory autoimmune diseases of the central nervous system. The Department of Neuropathology is associated with an international Graduate School for MD-PhD in Neurosciences and participates in an international collaborative effort supported by the National MS Society of the Unites States to define the pathology of the MS lesion (The MS Lesion Project).

The principal investigator: Wolfgang Brück, MD, Chairman of the Department of Neuropathology of the University of Göttingen. He has been working in the field of neuroimmunology for 15 years. The main focus of his work is the immunopathology of the MS lesion and its clinical, radiological and immunological correlates. He is also experienced in animal models of MS including EAE and cuprizone-induced demyelination and remyelination.

Research group members involved in the project:
Christine Stadelmann, MD, works in the field of neuroimmunology since 10 years and has extensive experience in analysing animal models of MS, immunohistochemical and molecular methods.
Fatima König, MD, postdoctoral fellow in the Department of Neuropathology is part of the MS lesion project. She has extensive experience in the pathology of MS and is responsible for collecting all the clinical data available from biopsied MS patients to be analysed in the planned experiments. She possesses profound clinical and radiological knowledge.
Imke Metz, MD, postdoctoral fellow, has clinical training in neurology and neuropathology and is involved in clinical studies as well as characterization of MS pathology.
Uta Scheidt, is a trained technician (>20 yrs), expert in the field of immune histology, microscopy and protein analysis.

Five relevant publications:

P3/ULUND
The institution: Lund University is the largest university in Scandinavia. The department of Medical Inflammation Research (MIR) (http://net.inflamm.lu.se/), is located at the Biomedical Center, Lund University Medical Faculty, Sweden and is headed by Holmdahl. The department has extensive experience in studies of experimental models for chronic inflammatory diseases like multiple sclerosis and rheumatoid arthritis.

MIR has a lab space of 850 m² and an animal house with a space of 650 m². The animal house contains 15000 mice and rats. A large number (>200) of unique mouse and rat strains are bred, genetically monitored and analysed here. These are selected (appropriate genomes) or made (transgenics) specifically for studies on models for MS and other inflammatory diseases. Mice are only incorporated via embryo transfer. The animal technicians work integrated in the research group. Beside this there is a well equipped laboratory containing a modern histopathology lab with computerised image analyses, a molecular biology lab including robotics and a microcapillary elphoresis (Megabace) work station and microarray reading and spotting equipments, a biochemical lab and a cellular immunology lab. The Medical Inflammation Research lab is an EU granted Marie
Curie training lab on animal models for inflammatory diseases which involve training program for PhD students from all over Europe.

The principal investigator: Rikard Holmdahl, Professor, head of MIR. To be directly involved in the project part-time (50%). Will be the principal investigator and partner in the cooperation.

Research group members involved in the IP and their experience:

1. Ragnar Mattsson, Professor, head of the transgenic resource center at Lund University. Expertise in embryo transfer, genetic targeting technology and the creation of genetically designed mouse strains. To be directly involved parttime (20%).
2. Thomas Blom, assistant Professor, director of animal technology, bioinformatics and phenotyping at MIR. (9 years experience) (20%). Will be responsible for the database for calculations of geno-phenon and pheno-phenon interactions.
3. Åsa Andersson, associate Professor, immunologist and geneticist, expertise in experimental animal techniques, immunology and animal genetics (10 years experience) (50%). Will be responsible for the genotyping and sequencing work of the animal platform.
4. Anna Karin Lindqvist, postdoc, expertise in mouse models and genetic analysis (6 years experience) (50%). Will be responsible for statistical evaluation and interaction analysis using R package and the Arexis database. Will be responsible for genetic pathway analysis and comparative genetics.
5. Lena Wester, postdoc, expertise in rat MS models (5 years experience) (100%). Will lead the rat genetic work.
6. Martina Johannesson, postdoc, expertise in genetic analysis of mouse EAE models (5 years experience) (100%). Will lead the mouse genetic work.
7. Three animal technicians specially trained for animal care, disease monitoring and embryo transfer (100%). Will have the practical responsibilities for running the animal unit.
8. Three laboratory technicians specially trained for genotyping, sequencing, biochemistry, phenotyping like expression analysis, proteomics, flow cytometry, immunosassays and histopathology (100%).

Five relevant publications:

The institution: The Division of Neuroimmunology of the Center for Brain Research is located in the Medical University of Vienna, Austria. The Brain Research Center has been created in 1999 and contains divisions for Neuroimmunology, Neurophysiology, Biochemistry and Molecular Biology, and Cellular Neurobiology. It has been newly equipped with the infrastructure for basic research. The division of Neuroimmunology has been working mainly on immunopathology of human inflammatory diseases of the nervous system and of respective experimental animals models. It is since more than 20 years engaged in international collaborations on different aspects of immune surveillance of the nervous system and the mechanisms of inflammation induced tissue damage. In addition a large collection of brain biopsy and autopsy material has been collected in the Division during the last decades, including the world’s largest collection of archival autopsy material from multiple sclerosis patients. The techniques, required for this project, including general neuropathology, immunocytochemistry, confocal laser imaging, electron microscopy, in situ hybridization as well as extraction of DNA from archival autopsy tissue in well established within the laboratory.

The principal investigator: Prof. Dr. Hans Lassmann is acting as Director of the Center of Brain Research of the Medical University of Vienna. He is working in the field of multiple sclerosis research since more than 30 years. His major achievements are a new classification of MS lesions, detailed studies on immune effector mechanisms involved in demyelination and axonal injury in MS as well as experimental studies on the mechanisms of inflammation and tissue injury in inflammatory brain diseases. He has been involved in several previous EU-funded research projects and is Coordinator of a current EU-Project on “Mechanisms of Brain Inflammation”. His expertise is in the field of immunopathology. Currently his core group consists of two senior scientists, 1 Post-Doctoral Scientist, 2 PhD students and 2 Technicians. He will spend 20% of his time on the project.

Research group members involved in the IP and their experience:
Dr. Jan Bauer (PhD) is a senior scientist in the group. His expertise is in the filed of immunocytochemistry, confocal laser imaging and electron microscopy. He has a profound training in human and experimental neuropathology, in particular in the filed of inflammatory brain diseases. He will, spend 20% of his time on this project.
Dr. Johannes Berger (PhD) is a senior scientist in the field of molecular biology. He has ample experience on DNA and RNA extraction of archival biopsy and autopsy material and PCR-technology. He will, spend 10% of his time on this project.
Mrs. Marianne Leiszer and Mrs. Helene Breitschopf are trained technicians, experts in the filed of general human neuropathology, immunocytochemistry, confocal laser imaging and electron microscopy, in situ hybridization as well as extraction of DNA from archival autopsy tissue in well established within the laboratory.

Five relevant publications:
1. Lassmann, H Brain damage when multiple sclerosis is diagnosed clinically. Lancet. 2003 Apr 19; 361(9366): 1317-8
2. Lassmann, H; Reindl, M; Rauschka, H; Berger, J; Aboul-Enein, F; Berger, T; Zurbriggen, A; Lutterotti, A; Bruck, W; Weber, J; Ulrich, R; Schmidt, M; Jellinger, K; Van de Veer, M A new paraclinical CSF marker for hypoxia-like tissue damage in multiple sclerosis lesions. Brain. 2003 Jun; 126(Pt 6): 1347-57

P5/MUW

The principal investigator: Prof. Dr. Hans Lassmann is acting as Director of the Center of Brain Research of the Medical University of Vienna. He is working in the field of multiple sclerosis research since more than 30 years. His major achievements are a new classification of MS lesions, detailed studies on immune effector mechanisms involved in demyelination and axonal injury in MS as well as experimental studies on the mechanisms of inflammation and tissue injury in inflammatory brain diseases. He has been involved in several previous EU-funded research projects and is Coordinator of a current EU-Project on “Mechanisms of Brain Inflammation”. His expertise is in the field of immunopathology. Currently his core group consists of two senior scientists, 1 Post-Doctoral Scientist, 2 PhD students and 2 Technicians. He will spend 20% of his time on the project.

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2. Lassmann, H; Reindl, M; Rauschka, H; Berger, J; Aboul-Enein, F; Berger, T; Zurbriggen, A; Lutterotti, A; Bruck, W; Weber, J; Ulrich, R; Schmidt, M; Jellinger, K; Van de Veer, M A new paraclinical CSF marker for hypoxia-like tissue damage in multiple sclerosis lesions. Brain. 2003 Jun; 126(Pt 6): 1347-57

P6/INSM

The institution: The group of Neuroimmunology of INSERM Unit 563 is located in the Purpan School of Medicine, Toulouse III University. It is part of the larger Pathophysiology Research Institute of Purpan University Hospital campus. The laboratory has been fully reshaped and renewed 1 year ago and possesses all the equipments necessary for tissue culture, FACS analysis, ELISA and molecular biology. In addition, the Institute possesses a state-of-the-art animal facility growing and maintaining specific pathogen-free mice and fully authorized for the generation and maintenance of transgenic lines. The Neuroimmunology group has expertise in the generation of transgenic mice, in cellular immunology, in the labelling and tracking of lymphocyte subpopulations in vivo/ ex vivo and in the evaluation of lymphocyte functions in vitro, both at the cellular and molecular levels.

The principal investigator: Prof. Liblau is the head of the Neuroimmunology Group at the INSERM Unit 563, Toulouse. He has worked for nearly 15 years in the fields of organ-specific autoimmune diseases and Neuroimmunology. His group has developed several novel models of autoimmune diseases using transgenic mice. These models have shed new light regarding the respective role of different subpopulations of autoreactive T cells (Th1 vs Th2, cytotoxic CD8 T cells) in the pathophysiology of these diseases. This has led to new therapeutic concepts for the treatment of human diseases. He has been involved in a large number of international collaborations, both within and outside Europe, leading to collaborative scientific publications. Among these collaborative efforts, recent work has been completed with several partners of the present consortium. Prof. Liblau is the leader of the EFNS Neuroimmunology scientist panel; he is a co-founder of the European School of NeuroImmunology (ESNI), and is a member of the scientific board of the French MS society (ARSEP). He will spend 50% of his time on the project.

Research group members involved in the IP and their experience:

Leonardus Mars, Post-doctoral fellow with more than 6 years expertise in the field of experimental models of autoimmune diseases. He has strong expertise in cellular Immunology and analysis of humanized mouse models.

Jacques Zappulla, Post-doctoral fellow with expertise both in Neuroscience and Immunology. He has strong expertise in the generation and analysis of genetically-modified mice (transgenesis, knock-out and knock-in mice).

Denis Bruniquel, Post-doctoral fellow with >6 years experience in molecular and cellular Immunology.

David Brassat, Neurologist and PhD student, has strong expertise in the field of MS genetics and in the analysis of experimental encephalomyelitis.

Cécile Cassat, Ingenier and PhD student, with >2 year experience in cellular Immunology.

Nicolas Couturier, Master student.

Sabine Desbois, Laboratory technician with >5 years experience in analysis of animal models of multiple sclerosis, cellular and molecular Immunology, tissue culture, FACS analysis.

Five relevant publications:


P7/MPG

The institution: The Max-Planck-Institute of Experimental Medicine in Göttingen (Germany) carries out basic and clinical research in neurosciences. The institute consists of 6 independent departments and research groups with more than 250 employees. Central facilities include state-of-the-art proteomics, electron microscopy, 2P-confocal microscopy, and a transgenic facility. Local interactions exist with scientists of the University of Göttingen (including the Hertie Multiple Sclerosis Institute), the German Primate Research Center, the European Neuroscience Institute (ENI), and the Max-Planck-Institute of Biophysical Chemistry. Research is strengthened by a joint DFG center grant (CMPB) and an international PhD programme.

The principal investigator: Klaus-Armin Nave, Ph.D. is directing the Department of Neurogenetics at the MPI of Experimental Medicine, and is Professor of Molecular Biology at the University of Heidelberg. He has been
working on myelin proteins and mouse models of myelin diseases for 20 years. Contributions to the field include molecular cloning of major myelin proteins, establishment of transgenic disease models, and defining oligodendroglial support for axonal integrity. Current interests include molecular mechanisms of axon-glial interactions and experimental therapies of myelin diseases.

Research group members involved in the project:
Moritz Rossner, PhD, Research associate with 7 years of postdoctoral experience: neuronal gene expression analysis, laser-directed microdissection, Affymetrix-based gene expression analysis.
Michael Sereda, MD, Research associate with 6 years of postdoctoral experience, animal models of myelin diseases, experimental therapies, axon-glia interactions.
Wiebke Möbius, PhD, Postdoctoral fellow (4 years): electron microscopy, cryo-sectioning.
Annette Fahrenholz, Technician (11 years): histology, immunohistochemistry
Carolin Stuenkel, Technician (3 years), molecular biology, Affymetrix-based transcriptomics
Gudrun Fricke-Bode, Technician (8 years): primary cell cultures.

Five relevant publications:

P8/KI
The Institution: KI is the Medical Faculty of Stockholm, Sweden. About 40 % of the biomedical research in Sweden is done at KI. We are located at the Center for Molecular Medicine, Karolinska Institute. We have long term experience in rat models for MS and neurodegeneration. In particular, we have spent the last ten years in intense efforts in dissecting genome regions regulating these models. In these efforts, we uniquely possess an advanced intercross line (AIL) between susceptible and resistant rat strains permitting gene localization down to 1-10 genes. We have also developed a large series of rat strains congenic for disease regulating regions, of importance for experimental pharmaco-genomic studies. By comparative genomics, these can be studies for relevance in large materials of persons with MS and population based controls. Our group has long term experience in studies of candidate genes in MS.
Our Institute is fully equipped with cutting edge technology for molecular biology, high throughput genotypings, animal house, immunology, histology/confokal microscopy.
The principal investigator: Tomas Olsson, to spend 50 % of his time on this project, is professor in molecular medicine and senior staff physician in neurology, head of the section for CNS research (Clinical Neurology, neurosurgery, neuropsychology, neuroradiology). He has 25 years experience in both clinical and experimental Neuroimmunology. He has previous experience as partner in two former EU supported concerted actions, and coordinator of one.
Research group members involved in the IP and their experience:
Professors/senior scientists/post docs:
Jan Hillert, professor in Neurology, 20 years experience in MS genetics, and has co-ordinated one previous EC project on MS genetics
Ass prof Fredrik Piehl, expertise in experimental neuroscience/neurodegeneration and its genetics.
Ass prof Bob Harris, expertise in immunology and protein chemistry.
Associate professor Erik Wallström, expertise in clinical and experimental Neuroimmunology/genetics
Ass prof Lou Brundin, expertise in neural stem cells, NO metabolism
Ass prof Magnus Andersson, expertise in Neuroimmunology
Kristina Becanovic, expertise in rat genetics
Maja Jagodic, expertise in rat genetics
Olle Lidman. expertise in genetics of neurodegeneration
Margarita Diez, Thomas Masterman, Anna Fogdell-Hahn, Helena Modin, all experts in MS genetics.
Two animal technicians trained for animal care and disease monitoring.

Five relevant publications:
FP6-2004-LIFESCIHEALTH-5  Integrated Project  NeuroproMiSe


**P9/NPHI**

**The institution:** The National Public Health Institute (KTL) of Finland is one of the most prestigious research institutes in biomedicine in Europe. The Department of Molecular Medicine of KTL is located in the brand new research building, Biomedicum Helsinki, next to the University hospital. This new, five storey 24,000 net m² research building houses about 1200 research employees and over 200 research groups from several organizations. This exceptional and interactive research environment is equipped with state of the art instrumentation which is readily shared by groups. To facilitate modern, cutting edge biomedical research, the Biomedicum Helsinki has had special emphasis to develop core facilities and research services. Relevant facilities for this proposal include: 1) Immediate vicinity to the University Hospital and equipped appointment rooms for research subject visits. 2) A large scale DNA extraction and storage facility which presently houses DNA from more than 200,000 individuals and is equipped with state of the art liquid handling robots, storage facilities, an automated extraction equipments. The database management is harmonized between the DNA sample core, sequencing and genotyping units. It provides services on a subsidized recharge basis. 3) The genotyping facility, Finnish Genome Center (FGC www.genome.helsinki.fi) is an independent department of the HU and provides high throughput genotyping services. FGC has a staff of 20, including senior scientists, graduate students, technicians and administrative staff. 4) The Biomedicum Biochip Center (BBC www.bioinfo.helsinki.fi) provides microarray core facility services for both custom made and Affymetrix arrays. 5) The Biomedicum Bioinformatics Core (BBU) is a joint operation between HU and KTL. BBU provides biocomputing expertise as well as computing resources for researchers and their collaborators to perform bioinformatics analysis.

**The principal investigator:** Dr Leena Peltonen (Palotie) is one of the most recognized human molecular geneticists worldwide. Her major contribution has been in the identification and characterization of human disease genes and dissection of the details of molecular pathogenesis resulting from mutations in these genes. Since 1994 she is Professor of Medical Genetics and Molecular Medicine in the University of Helsinki and the National Public Health Institute, Finland. In 1998 Dr. Peltonen was invited to the University of California, Los Angeles (UCLA), where she established a Genetics research Center with a wide spectrum of research activities truly reflecting all the aspects of the “post-genome”-era. These activities have gained her the recognition in the form of the “Gordon and Virginia MacDonald Distinguished Chair in Human Genetics. In 2002 Dr. Peltonen returned to her home country Finland to lead a major European research program, Genomewtwin, which studies large twin cohorts of Europe and aims to characterize genetic and lifestyle risk factors behind common diseases. Currently Dr. Peltonen has a prestigious Academy Professorship and she heads a Center of Excellence of the Academy of Finland as well as one of the Nordic Centers of Excellence in Genomics. Dr. Peltonen has published over 370 original publications and supervised over 50 Ph.D. students.

**Research group members involved in the IP and their experience:**

Dr. Janna Saarela, M.D.Ph.D., research scientist with six years of experience in MS research. Molecular genetics, statistical analyses, high throughput SNP genotyping.

Dr. Pentti Tienari, M.D.Ph.D. senior scientist, specialist in neurology, over 10 years of experience in clinical and basic research. Clinical research of MS, epidemiological research of MS, genetic studies of MS.

Dr. Anu Loukola, Ph.D. research scientist with four years of postdoctoral experience in high throughput genotyping and genetic studies of complex traits.

Suvi Kallio, M.D.Ph.D. students, graduate student with two years of experience in genetic studies of MS.

Denis Bronnikov, Ph.D. student, a graduate student with four years of experience in genetic studies of MS.

Five relevant publications:


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**P10/USOU**

The Institution: The CNS Inflammation Group has its laboratories in the School of Biological Sciences at the University of Southampton. Within the University there is broad experience in the field of immunology and in particular in the areas pertaining to autoimmune disease and oncology. Neurosciences are represented across the Faculty and focus on the areas of neuroinflammation, neurodegeneration and synaptic signalling. The School of Biological Sciences has state-of-the-art Bio-imaging facilities, high throughput proteomics facility and Biomedical research facility for the generation and maintenance of transgenic animals.

The principal investigator: Hugh Perry is Professor of Experimental Neuropathology, Head of the CNS Inflammation Group, and Director of the Southampton Neuroscience Group. He has worked on inflammation biology in the central nervous system for more than 20 years. The focus of this work has been to understand the role that inflammation plays in acute and chronic neurodegeneration. He has investigated the molecular mechanisms that underpin axon degeneration and the mechanisms by which inflammatory cells damage axons and induce their degeneration. The laboratory uses a broad spectrum of animal models to study different disease processes and a spectrum of different techniques (immunocytochemistry, ELISA, RT-PCR, Taqman PCR, Western blotting etc) to evaluate cellular and molecular inflammatory processes involved in neuronal injury and degeneration.

Research group members involved in the IP and their experience:

Dr Tracey Newman - Research Scientist - mechanisms of axon pathology (immunocytochemistry, imaging)

Dr Colm Cunningham - Research Scientist - inflammation in chronic neurodegeneration (behaviour, cytokine biology)

Dr Cailong Fang - Research Scientist - mechanisms of axon pathology (Wld mutation, biochemistry, immunocytochemistry)

Dr Martine Bernardes-Silva - Research Scientist - blood-brain barrier biology (biochemistry, immunocytochemistry, imaging)

Dr Ian Galea – Research Scientist/Neurologist – CD8 T-cell induced pathology (immunocytochemistry, imaging, cell culture)

Dr Leigh Felton – Research Scientist – peripheral immune brain communication (behaviour, cytokine biology, telemetry)

Mrs Sara Waters – Histopathologist/Laboratory manager

Mrs Penny Force – Histologist

Ms Suzzanne Campion – Research assistant

John-Paul Jukes – Research assistant

Bryony Gray – PhD student – synaptic degeneration in chronic neurodegeneration (biochemistry, immunocytochemistry, proteomics)

Emma Rankine – PhD student: axon degeneration (biochemistry, proteomics)

Roxanne Nnardi – PhD: CNS drainage pathways (tracers, imaging)

Five relevant publications


P11/KCL

The Institution: King’s College London is the largest medical school in Great Britain, formed by the amalgamation of three of the most famous British medical institutions, namely the medical schools of Guy’s, King’s and St. Thomas’ (GKT) Hospitals. Located in the heart of London, at London Bridge, the GKT School has 3,000 full time academic members of staff, and it is ranked in the top five UK academic research institutions (of approx. 200). The institution has all the facilities expected of a major international, research-led university.

The Principal Investigator: Kenneth Smith, PhD, is Professor of Neurophysiology and Head of the Neuroinflammation Research Group. His focus of research is the mechanisms underlying the neurological deficits in disorders characterised by inflammation, demyelination and/or degeneration. The main focus is multiple sclerosis (MS), but his research also includes projects on Guillain-Barré syndrome (GBS), motor neuron disease, glaucoma and the overactive bladder. Particular current interests are the several roles of nitric oxide in neuropathology, and developing a novel therapeutic strategy for axonal protection in neuroinflammatory disease such as MS. On-going techniques include extracellular and intracellular electrophysiology, patch voltage clamp, immunohistochemistry and confocal microscopy. The group currently includes 12 researchers, 5 of whom are clinically qualified and 7 are qualified in the basic sciences, with training in electrophysiology, morphology, pharmacology and pharmacy. Apart from colleagues in the current application, active research collaborations include projects with Aventis, USA; GlaxoSmithKline, UK; Organon International, The Netherlands; Pfizer, USA; the Salpetriere Hospital, Paris; UCB, Belgium; University College, London; and Yale University, USA.

Research group members involved in the project:
Ms Rebecca Collinson, BSc. Research assistant. Histological techniques, immunohistochemistry, electron microscopy.
Dr. Pallab Seth, PhD. Electrophysiology. Role of nitric oxide in axonal pathophysiology
Dr. Richard Evans, PhD. Electrophysiology. Therapies to modulate conduction in demyelinated axons
Dr. Marianne Kasti, MD. Experimental neuropathology. Immunohistochemistry. Role of energy depletion in neuroinflammatory disease
Ms. Clare Farmer, BSc. PhD Student. Biophysics; patch clamp. Effects of inflammation on neuronal biophysics
Dr. Arun Sahai, MD. PhD Student. Experimental neuropathology. Immunohistochemistry. Botulinum therapy of overactive bladder
Dr. Marija Sajic, MD. PhD Student. Experimental immunology. Flow cytometry. T cell biology. Role of CD8 T cells in multiple sclerosis
Mr. Oskar Jachimczuk, BSc. Research Assistant. Pharmacology. Sodium ion pathways in myelinated nerve fibres
Miss. Hannah Morgan, BSc. Research Assistant. Behavioural testing. Role of NO in neuropathic pain
Dr. Yue Sun, MD. Research Assistant. High resolution microscopy. Histological investigation of neuroinflammatory lesions

Five relevant publications:

P12/USTUTT

The Institution: University of Stuttgart is one of the leading German universities in engineering and natural sciences and was recently ranked among the top German universities in the field of biological research (based on DFG grant acquisition). The diploma study course of Technical Biology has a high reputation. The Institute of Cellbiology and Immunology (IZI) is a driving force in these research and teaching endeavours; it is part of an interdisciplinary Center of Bioengineering devoted to basic and applied sciences. The focus of research is in signal transduction, transgenic animal models of disease, biomedical research in oncology and in neuroimmunology.

The Principal Investigator: Klaus Pfizenmaier, PhD, Professor of Cell Biology, Director of IZI, profound experience in establishment and management of larger research consortia (directory board member of ZSP Bioverfahrenstechnik Stuttgart, a bioengineering consortium comprised of 25-30 groups, 1992-2000; founder and chairman of the DFG Collaborative Research Center 495 (19 groups), 2000-date, participant of two EC projects in the Biotechnology programme 1994-1998; Dean of the Faculty for Geo- and Biosciences at the University of Stuttgart, 1996-1997. More then 25 years of active research with >180 publications in the field of
cellular immunology, cell biology, cytokine function and signal transduction mechanisms; development of TNF based therapeutics, patents and several patent applications relevant to the proposal.

Research group members involved in the IP and their experience:

Peter Scheurich, professor for Immunology, Vize-chairman of IZI, since 15 years actively working in the field of TNF intracellular signalling and apoptosis, ~100 publications. Current focus of work on the functional role of TNF and the two defined TNF receptors. Co-leader of an interdisciplinary group working in systems biology. Several patents/patent applications relevant to the proposal. Co-chairman of the DFG Collaborative Research Center 495.

Roland Kontermann, professor for biomedical engineering, since >10 years working in the field of antibody engineering, phage display technology and targeted therapeutics, >40 publications and book chapters. Current focus of work on the generation of recombinant antibodies and targeted liposomes for cancer therapy. Several patents/patent applications. Co-editor of the lab manual "antibody engineering".

Anja Krippner-Heidenreich, PhD (>4yrs postdoc) molecular biologist, TNF signal initiation and transduction mechanisms by the two TNF receptors.

Lara Marchetti, PhD (<4yrs), molecular and cellular neurobiologist, profound experience in transgenic animal models, primary neuronal cultures, excitotoxicity models, neuronal cytokine signal pathways, viral vectors, in vivo models of neurodegeneration.

Jeanette Gerspach, PhD (<4yrs), generation of TNF fusion proteins, in vivo models, pharmacokinetic and immune histology studies.

Marcus Branschädel, Ph.D. student, TNF and CD40 intracellular signaling pathways.

Sabine Müntel, Dipl.Ing.Biotech (>10 yrs), eukaryotic expression systems, lab scale production of recombinant proteins, in particular TNF fusion proteins, biochemical purification.

Gudrun Zimmermann, tech assist. (>10 yrs), biological assays of cytokine function and molecular biology techniques.

Nathalie Peters, tech. assist. (>5yrs), molecular biology, tissue culture and microscopy.

Five relevant publications:


Patents:

P13/USGR

The institution: The Groningen University is typically embedded in the Dutch university system in which research activities and training programs are highly interacting. The Groningen team of this IP is a group of members of the Faculty of Biology from the fields of pathophysiology and molecular and cellular neurobiology. They have extensive experience with in vivo model study of neurodegeneration and neuroprotection, investigating basic mechanisms of brain plasticity in health and disease, and testing newly developed drugs, neuroactive hormones like corticosteroids and estrogens, and food supplements. Recent extension of the team includes molecular neurobiology expertise on neuroinflammatory mechanisms and the generation of genetically modified cells and mice with up-to-date technology. The expertise of P13 can be characterized as the combined structural (neuroanatomy, receptor localization, electronmicroscopy, neuronal and cerebrovascular integrity) and functional (extensive behavioural analysis) in vivo evaluation of neuroprotective strategies in rodent models that mimick aspects of aging and Alzheimer’s disease, glutamate excitotoxicity and hypoxia, and cerebral ischemia.
Neuroprotective approaches are carried out on live animals, organotypic culturing of brain slices and primary neuronal cultures.

The principal investigator: Ulrich Eisel, PhD, assistant professor of Molecular Neurobiology, with extensive and more than 13 years lasting experience in molecular neurobiology and the generation of transgenic and mutant mouse models in the fields of neurotoxicity, neuroinflammatory responses and NMDA receptor biology. As a former member of the Institute of Cell Biology and Immunology in Stuttgart, he gained great experience in the field of Tumor Necrosis Factor signalling in the central nervous system in disease situations and he was participating in a former EC project “Modelling human neuroinflammatory diseases in transgenic and mutant mouse models”. In the new position in Groningen he has established in the Department of Molecular Neurobiology a group for the generation of transgenic and mutant animals and the molecular and cellular investigation of TNF signals. He is also cooperating with the other members of the group on various projects. One U.S. patent application on a mouse model in neurodegeneration relevant to the proposal is submitted.

Research group members involved in the IP and their experience:

Prof. Dr. Paul G. Luiten, professor of Molecular Neurobiology, head of the department, Director of the Research School of Cognitive and Behavioral Neuroscience (CBN) is author or co-author of 180 international publications. He has extensive experience in the field of Alzheimer Research, Dementia, vascular diseases of the brain and long term experience in co-operative research with the pharmaceutical industry in neurodegeneration and neuroprotection.

Prof. Dr. C. Nyakas, part-time professor of Pathophysiology in Groningen and fulltime professor in Pathophysiology at the Semmelweis University, Budapest, with great experience in the pathology of Alzheimer's Disease in humans and rodent models.

Dr. Eddy van der Zee, assistant professor of Molecular Neurobiology, has a long standing interest and profound expertise in the behavioral analyses and molecular neuroscience of rodents especially in the field of learning and memory.

Dr. Frans Maes, associate professor of Biophysics and a specialist in informatics, is also a training supervisor.

Mrs. Amalia-Mihaela Dolga, PhD student, Ubbo Emmius stipend, former Erasmus exchange program student from Romania in Bremen, with great experience in cellular neurobiology and molecular biology.

Mr. Jan Keijser, head technician with a long experience (~23 years) in receptor analysis, molecular biology, image analysis, and informatics.

Mr. Jan Gast, technician cell culturing, histology

Mrs. Josée Plantinga, trained technician with 5 years of working experience in in vitro fertilization, microinjection, in situ hybridisation, Immunostaining, tissue culture, transgenics, and molecular biology.

Mr. Jaap Bouwer, animal caretaker, breeding programs

Five relevant publications:


P14/HPI

The institution: The Hellenic Pasteur Institute has an 83-year history in the field of biomedical research focused on the study of infectious diseases, immunology and neurobiology as well as on several aspects of public health. Research activities are carried out in 11 independent research laboratories organised into two Departments (Microbiology and Biochemistry). The experimental animal and confocal microscopy units operating in the HPI offer to the researchers the possibility of extending their studies in important research areas with the use of transgenic animals as in vivo models of human diseases. A principal target for the HPI is the exploitation of research findings towards the development of novel therapeutic approaches, the production of new-generation vaccines, and reliable diagnostic methods with increased specificity and sensitivity.

The principal investigator: Lesley Probert, PhD, Head of the Laboratory of Molecular Genetics and Scientific Director of the Experimental Animal Facility of the Hellenic Pasteur Institute. She has more than 15 years experience in the fields of cellular and molecular neuroimmunology, and in the development and study of...
transgenic mouse models of CNS neuroimmune disease. Member of the scientific committee and course organizer/ instructor for the European School of Neuroimmunology (ESNI). Coordinator of EU-funded research project BIO4CT-960174 entitled “Modeling human neuroinflammatory and demyelinating disease in transgenic and mutant animals” under the BIOTECH 1994-1998 programme, which also involved present partners P5, P13 and P13. The laboratory is fully equipped to cover most methodologies in molecular and cellular biology, immunology and neurobiology and actively invests in the development and application of emerging technologies in the field of functional genomics (cDNA microarrays, bioinformatics and transgene-based RNAi for gene knockdown in mice, advanced flow cytometry analyses). The laboratory runs its own transgenic technology unit and breeding station for the development and distribution of transgenic disease models and mutant mouse strains.

Research group members involved in the project:
Dr Sylva Haralambous – Research Engineer – inflammation biology and the role of inflammation in acute and chronic tissue destruction. Responsible for laboratory’s transgenic unit (immunochemistry, cell culture, transgenic technologies, siRNA).

Dr Era Taoufik – Postdoctoral Research Scientist – TNFR signaling mechanisms, neurodegeneration and neuroprotection mechanisms (molecular biology, gene expression technologies, viral vector engineering, siRNA, biochemistry, cell culture, mouse neurodegeneration models).

Dr Vivian Tseveleki – Postdoctoral Research Scientist – TNFR signaling mechanisms, T cell biology, gene expression analysis of CNS immune-mediated disease (molecular biology, immunology, microarray analysis, bioinformatics and algorithm development, FACS, EAE).

Miss Stavroula Lambropoulou – Research assistant, transgenic breeding programmes and disease monitoring.

Miss Maria Evagelidou – PhD student – role of caspase 8 in cell proliferation (gene expression technologies, biochemistry, cell culture, EAE).

Miss Mary Emmanouil – PhD student – conditional expression systems for TNFR1 signaling pathway in mice (conditional gene expression in mice, EAE).

Mr Angelos Vagionas – animal caretaker.

Five relevant publications:

P16/HBT
The Institution: Hbt is a biotech company (founded in 1994) producing recombinant proteins, monoclonal and polyclonal antibodies and ELISA kits for research laboratories engaged in immunology, endotoxin & other microbial toxins, cytokine and chemokine studies, cell-biology, pathology and cell and tissue damage research.

Hbt’s aim in research is developing antibodies and assays that fulfill the need and demand of the research community. Products are being developed and commercialized in full cooperation with Universities and research institutes worldwide. Hbt has obtained formal accreditation for its quality managements systems by obtaining ISO 9001-2000 certification. Main products are antibodies, proteins and assays for cytokine and chemokine studies; research of endotoxin & other microbial toxins; TLR and cell and tissue damage research. The major products for cytokine and chemokine studies: complete range of antibodies against TNF and TNF-R and recombinant proteins. Hbt will provide its production capacity to the project in order to supply the partners with the necessary reagents.

The principal investigator: Wim Buurman, PhD, professor of immunology has extensive experience in research in the field of cytokines, inflammation and innate and adaptive immunity with more than 300 publications. He has been partner in several research consortia and has organized several congresses Wim Buurman, technical director of Hbt, will co-ordinate Hbt’s activities in this project. Mr. W.H. de Niet, director of Hycult Biotechnology, Uden, is the legal representative. Gaby Francot, general manager, supports him.

Research group members involved in the IP and their experience:

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The principal technical staff is led by Mrs Gaby Francot (engineer in microbial sciences). Further two technical engineers will participate in the project: Marja van Genugten and Brenda Steenbakkers. They all have a permanent position. They have experience with the development, production and purification of recombinant proteins and antibodies (monoclonal as well as polyclonal). Furthermore they have ample experience in the development of ELISA assays and the evaluation of these assays.

**P17/ARX**

**The institution:** Arexis AB, founded in 1999 by world leading scientists active in the field of animal genetics, is a drug discovery and development company focusing on metabolic and inflammatory diseases. Since August 2005, Arexis AB is owned by Biovitrum AB. Arexis is biology-driven with proprietary technology and a strong focus on product development. Arexis uses forward genetics for a high-resolution molecular dissection of causative disease mechanisms. This research strategy is generating a new clinical programme every year and continues to yield novel and proprietary drug targets, key to a more effective development of novel therapies. Arexis has built a strong pipeline comprising innovative pre-clinical and clinical projects and established a highly competent and focused organisation with significant industrial experience, closely collaborating with both academic and industrial partners. Arexis currently employs 25 full time staff of which the majority works in Research & Development. The laboratories are fully equipped with advanced technological instruments required to perform modern scientific research within the field of molecular biology and protein engineering. Animal experiments are performed in a central facility (EBM) that is run by the Göteborg University together with the University hospital. Key personnel at Arexis have extensive experience from world-leading pharmaceutical business and hands-on experience from managing highly complex, international projects. The company also has experience from participating in European research programmes, for example from *Eurome*, a FP5 project focusing on rheumatoid arthritis.

**The principal investigator:** Dr. Peter Olofsson is the project leader of the NCF1 project at Arexis (2 years postdoc). The aim of the NCF1 project is to develop new therapeutic treatments against autoimmune disorders like MS, RA and IBD. Olofsson got his PhD in the laboratory of prof. Rikard Holmdahl. The achievements of this project resulted in the identification of and classification of EAE and arthritis regulating genetic loci in animal models. Through positional cloning this work eventually also led to the positional cloning of Ncf1 as one strong regulating gene of arthritis and EAE. The genetic analysis was primarily performed in rats but the findings has recently also been confirmed in mouse models. Dr Olofssons main competence is in the fields of molecular genetics and animal models, but he has lately also obtained knowledge in pharmacotoxicology experiments needed for preclinical drug safety evaluation studies. Dr Olofsson will spend 50% of his time on the project.

**Research group members involved in the IP and their experience:**

Amelia Nilsson is a research scientist (3 years) with education in bioinformatics and genetic profiling. She will spend 50% of her time on the project.

Camilla Bernhardsson is a research scientist that will work mainly with animal experiments and phenotyping interpretation of pharmacological data from the experiments. She will spend 50% of her time on the project.

Dr. Andrew Browning is a medicinal chemist that is responsible for the compound development in the program. He will participate at 20% in the project to optimise compounds for preclinical development.

Annika Nerstedt is a Research scientist (20 years) with long experience in RNA extraction and gene expression profiling. She will spend 50% of her time on the project.

Dr. Thomas Svensson (>10 years) has long experience in bioinformatics, genetic profiling and data management. He will spend 10% of his time on the project.

Sofia Martinsson is a research scientist (5 years) with experience in molecular biology, protein expression/purification and tissue culture. She will spend 10% of her time on the project.

Elisabeth Nilsson is a research scientist (3 years) with education in bioinformatics and genetic profiling. She will spend 25% of her time on the project.

Dr. Löwenadler is the CSO at Arexis and is an experienced senior scientist with a background in molecular biology. Dr Löwenadler has more than 15 years experience of research management and coordination in biomedical research in the pharmaceutical industry. He has held positions as Head of Molecular Biology and Target Discovery at Pharmacia and AstraZeneca, respectively. Dr Löwenadler will be involved in the NCF1 project steering and preclinical development. He will spend 5% of his time on the project.

**Five relevant publications:**


**P18/ELL**

The institution: Eisai London Research Laboratories Ltd. (ELL) is a research-based drug discovery facility established in 1990 by Eisai Co., Ltd. Japan. ELL is located on the main campus of University College London (UCL). The research strategy is focused on drug discovery for neurodegenerative disorders using molecular and cell biology techniques. The research is focused on target finding, target validation and drug discovery for Multiple Sclerosis, Alzheimer’s disease and Parkinson’s disease. The facility consists of 4 research groups with 34 researchers supported by an administrative department. The scientists work in either one of two molecular cell biology groups, a pharmacology group or a medicinal chemistry group. The laboratory facilities provide modern technological instruments including Cellomics High Content Screening, mass spectrometers for proteomics, HPLC equipment for measurement of neurotransmitters, image analysis systems. The personnel in the company have extensive experience in several animal models of multiple sclerosis including the mouse Myelin Oligodendrocyte Glycoprotein (MOG) model and the Lewis Rat Experimental Allergic Eencephalomyelitis. We plan to use our location on the campus of UCL to foster collaborations with members of the physiology department to examine calcium signalling.

The principal investigator: Dr. Toshal Patel joined Eisai in 1999 and is the deputy leader of the pharmacology group. He has extensive experience in working with in vivo disease models and in the area of neuroprotection.

Research group members involved in the IP and their experience:

Dr. Anthony Groom has extensive experience in working with rat and mouse models of EAE. He has experience in histopathological analysis for assessing neurodegeneration and neuroprotection models of EAE. He has previously worked on a project assessing the neuroprotective potential of AMPA receptor antagonists in EAE. This project is currently in clinical development.

Mr. Jonathan Papworth has experience in working with the mouse MOG EAE model and also in immunoblotting techniques for the detection of proteins from in vivo tissue.

Relevant publication:


**P19/UNI-BONN**

The institution: The Institute of Reconstructive Neurobiology is a recently founded institute of the Medical Faculty of the University Bonn. The Institute of Reconstructive Neurobiology is supported by the Hertie-Foundation and is focused on basic neuroscience research and novel therapy strategies of neurological diseases. The institute is located in the LIFE & BRAIN University Center, a new building having several technology platforms including cellomics, transgenics, genomics and neurocognition. The institute is associated with a graduate study program in biomedicine.

The principal investigator: Harald Neumann, MD, Head of the Neural Regeneration Unit at the Institute of Reconstructive Neurobiology has extensive and more than 12 years lasting experience in cellular and molecular neuroimmunology, and in the field of neurotoxicity mediated via adaptive and innate immunity. As a former member of the Max-Planck-Institute of Neurobiology and the European Neuroscience Institute Goettingen he gained strong knowledge in the field of neuro-immune interactions. He has profound experience in establishment and management of research consortia (founder and member of the managing board of the Institute of Multiple Sclerosis Research co-funded by the Hertie-Foundation and University Goettingen).

Research group members involved in the IP and their experience:

Kazuya Takahashi, postdoctoral fellow (>4 yrs), MD, PhD, since 7 years actively working in the field of neuroimmunology and innate immune receptors. Current focus of work is on the functional role of TREM2 in the nervous system.

Massimiliano Stagi, PhD student (> 3yrs) confocal and 2-photon-imaging, molecular biologist, axonal transport analysis and mechanisms of axonal injury.

Philipp Gorlovoy, PhD student (> 2yrs), confocal and 2-photon-imaging, molecular biologist, FRET-analysis of GFP-tagged molecules in primary neurons.

Sadanand Gaikwad, PhD student, molecular biology, in vivo models, immune histology studies.

Jens Kuerten, tech assist. (>1 yr), neuronal culture and molecular biology techniques.

Christine Frank, tech. assist. (>1yr), molecular biology, tissue culture and microscopy.

Five relevant publications:


**P20/DIREVO**

**The Institution:** DIREVO is the leading company in the business of screening-based directed evolution and its application to biomolecules, specifically to biocatalysts for chemical, technical, industrial, scientific and pharmaceutical purposes. Furthermore, Direvo has developed a New Biological Entity (NBE®) Platform which allows the generation of novel enzymes with tailor-made specificities and activities for pharmaceutical or industrial applications.

**The principal investigator:** Dr. Kettling is co-founder of DIREVO and has been CSO of the company since its inception in 2000. In this function he is responsible for all research and development work of the Company including its intellectual property position. He established DIREVO’s technology platform for the screening-based directed evolution of proteins, and co-developed the NBE® technology (generation of novel sequence-specific proteases, New Biological Entities (NBE)). Under his guidance, numerous molecular biological and technical innovations have been invented, which have been fundamental for DIREVO’s technological leadership in the area of Directed Evolution and NBE®. He established several major industrial and academic collaborations, and implemented an internationally renowned Scientific Advisory Board at DIREVO.

**Relevant publications and patents:**


Annex-I – “Description of work”, Page 153 of 155
A2. SUB-CONTRACTING

A. The Coordinator intends to retain a Professional Accounting Firm, as a subcontractor consultant to assist in the accounting and administrative management of the EC contribution and in assembling the management report for each reporting period.

The Professional Accounting Firm will assist the coordinator in the following management activities:

1) in ensuring that the EU contribution is used in accordance with EU FP6 Financial Guidelines and that proper accounting and reporting procedures are implemented

2) in developing an archiving, data retrieval, economic, accounting, and financial reporting system to allow the Coordinator to be able to issue on a by-yearly basis the following reports to the Consortium:
   a. interim economic and financial statements on the use of funds;
   b. pro-forma and final statements for the reporting period;
   c. 18-month rolling forecasts for each reporting period;
   d. reconciliation statements versus budget;
   e. preparation of audit certificates
   f. selection of a certified public accountant to certify the cost statements for each reporting period.

The above objectives will be achieved through the development of an accounting interface allowing input into, and retrieval from the central administration general accounts of the accounting information related to the use of funds for the Project in a format exportable and usable with currently available spreadsheet applications. This will be done in collaboration with the Central Administrative Offices of the Coordinator.

3) in developing accounting and reporting guidelines for the Consortium as a whole and for the single participants, to ensure uniformity of accounting and reporting procedures;

4) in training the investigators of the consortium, especially the project leaders, on accounting principles and contractual rules of the consortium contract and in the preparation of audit certificates by all Contractors;

5) in coordinating the assembly of the economic and financial reports of the Consortium to the EU Commission for each reporting period. Contractors have been informed that under FP6 Financial Guidelines the cost of audit certificates is a direct eligible cost under the "Management of the consortium activities" (second subparagraph of the first paragraph of Article II.26232 and paragraph 4 of Article II.2 of Annex II (General provisions) to the FP6 model contract); and henceforth that if the audit certificate is provided by an external auditor or a competent public officer from an organization other than the contractor, it is also considered as a subcontract. We have henceforth assumed, in both the form A3 and the form of section B8, that Contractors with the AC, FC and FCF cost models will resort to subcontractors, i.e. external auditor or a competent public officer from an organization other than the contractor, for the preparation of audit certificates.

B. Participant P17/ARX intends to resort to subcontracting companies for the following activities: i) chemical synthesis of new potential drugs (Enamine, Kiev, Ukraina); ii) biodistribution studies of radiolabelled compounds (decision still pending); and iii), preclinical safety and toxicology studies of new compounds (RCC, Itingen, Switzerland). All these activities are essential for the development and use in clinical studies of novel drugs targeting the Ncf-1 pathways. The major reasons for subcontracting these activities are that ARX has no internal capacity for chemical synthesis and biodistribution studies, and that, according to regulations from FDA and EMEA, safety studies of potential therapeutic compounds are required to be performed by good laboratory practice (GPL) authorized labs.

A.3 THIRD PARTIES –

Not applicable
A.4 COMPETITIVE CALLS
Not applicable

A.5 THIRD COUNTRY PARTICIPANTS –
Not applicable