



Publishable Summary

Final Report

Grant Agreement Number: 278611

Project acronym: NEURINOX

Project title: NOX ENZYMES AS MEDIATORS OF INFLAMMATION-TRIGGERED NEURODEGENERATION: MODULATING NOX ENZYMES AS NOVEL THERAPIES

Funding Scheme: Collaborative Project

Date of latest version of Annex I against which the assessment will be made: 20 October 2016

Period covered From 01/01/2012 to 31/12/2016

Name, title and organisation of the scientific representative of the project's coordinator:

Vincent Jaquet, PhD., Department of Pathology and Immunology, University of Geneva

Tel: +41 22 379 4136

E-mail: Vincent.Jaquet@unige.ch

Project website address: <http://www.NEURINOX.eu>

Table of Contents

1.	Final Publishable Summary	3
1.1	Executive Summary	3
1.2	Description of the Project Context and Objectives	4
1.3	Description of the main S&T results/foregrounds	6
1.4	Potential impact	10
1.5	NEURINOX partners and contact	21

1. Final Publishable Summary

1.1 Executive Summary



NOX Enzymes as Mediators of Inflammation- Triggered Neurodegeneration: Modulating NOX Enzymes as Novel Therapies

Neuroinflammation is a key process associated with neurodegenerative diseases (ND). The underlying hypothesis of the NEURINOX project is that NADPH oxidases (NOX) are mediators of neuroinflammation and that NOX are promising drug targets for the development of therapies against ND.

NEURINOX focuses on several diseases with strong neuroinflammatory components and other autoimmune neuropathies, including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and mesial temporal lobe epilepsy (MTLE) and other rare autoimmune peripheral neuropathies (APN), such as recurrent Guillain-Barré Syndrome (rGBS) and chronic inflammatory demyelinating polyneuropathy (CIDP).

The project is a multi-disciplinary research programme with the following specific scientific and translational objectives:

- Identify NOX-dependent signalling pathways: to understand how, when and in which cells NOX control the neuroinflammatory component of ND, and to elucidate their role in the progression of neuroinflammation and ND.
- Provide proof of principle for NOX inhibition or activation in animal models.
- Identify redox-dependent biomarkers of disease progression and severity in human samples from patients affected by ND.
- To develop small molecules NOX inhibitors and activators as therapeutics.

NEURINOX has made significant contribution for developing *in vitro* and *in vivo* models of neuroinflammation, identifying redox-dependent pathways regulated by NOX in ND novel mouse models for studying cell specific expression of NOX2, innovative methods for specific ROS detection, and development of new specific inhibitors/activators of NOX enzymes. At the clinical level, new oxidative biomarkers were identified for ALS and MS patients.

Most relevant results were either published or are in preparation for publication in scientific journals. These results obtained during the last year of the project consolidated previous results and have focused on identification of (i) genes controlling NOX2-derived oxidative burst and (ii) testing NOX inhibitors and anti-neuroinflammatory drugs in animal models of ND and (iii) regulation of NOX expression and activity as well as downstream targets of NOX activity.

1.2 Description of the Project Context and Objectives

1.2.1 Description of the Project Context

The family of NADPH oxidases (NOX) contains seven members NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2. They are membrane proteins comprising 6 transmembrane domains (7 for DUOX1 and 2), which use NADPH and oxygen as substrates to catalyze the formation of superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2).

Each NOX isoform is characterized by specific mechanism of activation and tissue distribution. Under physiological conditions, NOX are essential mediators of host defense and different biosynthetic processes, including thyroid hormogenesis and formation of otoconia. Mutations affecting the NOX2 enzymatic system cause a disease known as chronic granulomatous disease, which is characterized by increased susceptibility to infections by certain pathogens. Interestingly NOX2 lack of function complex is also associated with the development of several autoimmune chronic inflammatory diseases. Thus absence of NOX-derived oxidants has pathological consequences, excessive oxidant production by NOX enzymes also contribute to oxidative damage observed in cardiovascular diseases, pancreatitis and CNS pathologies ([Nayernia 2014](#)).

The most frequent neurodegenerative diseases (ND) are Alzheimer disease (AD), Parkinson disease (PD), amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and multiple sclerosis (MS). Symptomatic treatments exist for PD and MS, but no disease modifying therapies are available for other ND. The symptoms of ND are largely due to the progressive impairment of neuronal function and their specificity depends on the neuronal cell types and brain regions affected. Several features are commonly shared in ND: abnormal aggregation of key proteins, spatio-temporal spreading of aggregated proteins, neuroinflammation, and oxidative modifications of macromolecules. MS differs from other ND, as it is an autoimmune disease characterized by infiltration of leukocytes in the brain and spinal cord, which destroy the myelin sheath and lead to secondary neuronal death. Neuroinflammation in other ND is mostly sustained by astrocytes and microglia. Similarly to inflammation, neuroinflammation is a primary response of the host aiming at removing harmful stimuli and initiating healing processes.

However in ND, the initiating factors are not removed and Neuroinflammation become detrimental to neuronal cells and contribute to ND progression. Identification of novel drugs controlling neuroinflammation represents a promising therapeutic approach for ND. As major source of oxidants in ND and potential regulators of neuroinflammation, NOX represent a novel and promising class of pharmacological targets for the treatment of ND. A role of excessive NOX-formed $O_2^{\cdot-}/H_2O_2$ and secondary oxidants derived from them in CNS pathology is known since the first report that NOX2 gene knockout mice are protected from brain ischemia ([Walder 1997](#)). Since then, several NOX isoforms have been documented in the CNS and numerous studies have pointed towards of role of NOX isoforms in AD, PD, ALS and MS (reviewed in [Sorce 2012](#); [Nayernia 2014](#)).

Increased oxidative stress resulting from increased oxidant formation is a central feature of ND. NOX-derived oxidants can directly impair neuronal function due to their toxic properties, but they are increasingly recognized as key modulators signaling pathways potentially controlling neuroinflammation. Although oxidants are key components of ND, therapeutic approaches targeting reactive oxygen species have so far been ineffective. Lack of efficacy of antioxidant strategies may be at least partly due to lack of specificity *in vivo* efficacy and potential concomitant attenuation of the regulatory role of oxidants. Our approach consists in targeting a *primary source* of $O_2^{\cdot-}/H_2O_2$ (i.e. NOX) rather than 'scavenging' oxidants after they have been formed.

The NEURINOX concept

NOX present paradoxical features in ND: NOX-derived $O_2^{\cdot-}/H_2O_2$ lead to oxidative damage and resulting pathology, or are beneficial by regulating key physiological functions, including the resolution of inflammation in autoimmune diseases such as MS. Thus, depending on the pathology, the therapeutic approach should either enhance or mitigate NOX activity (**Figure 1**).

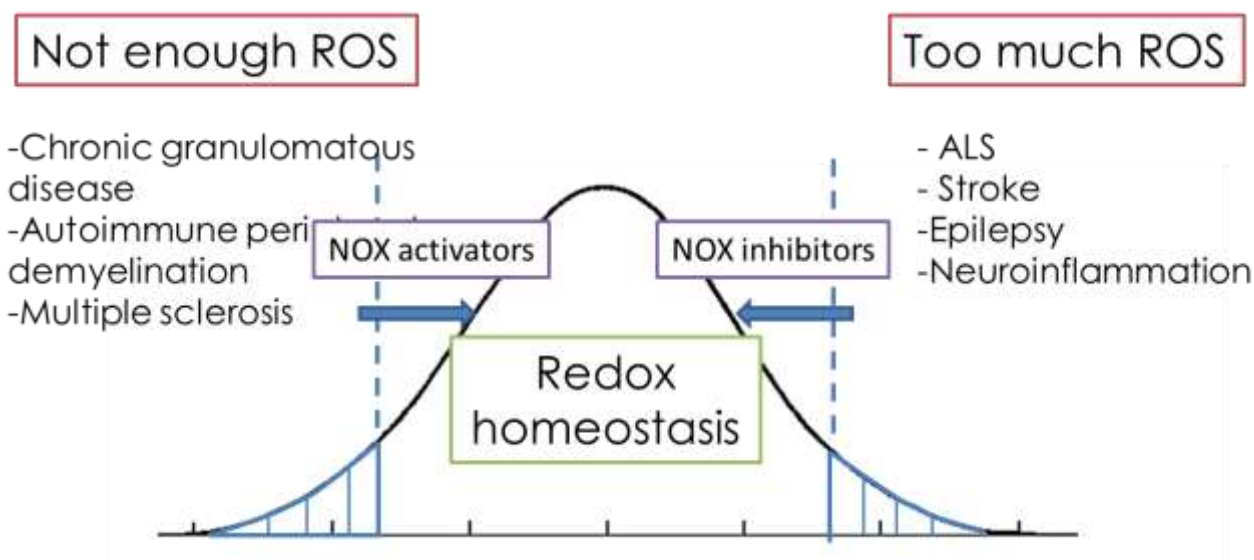


Figure 1: NOX-dependent ROS generation in general population follows a Gaussian distribution, with pathology at both extremes. The arrows represent the proposed therapeutic approach of NEURINOX to reach normal redox function.

Based on this concept, the NEURINOX consortium was formed in 2012 (<http://www.NEURINOX.eu>). It received funding from the European commission for 5 years and comprised researchers specialized in NOX, oxidants, inflammation and ND from the academic as well as clinical and industry settings.

1.2.2 Objectives

The principal objectives of NEURINOX were to:

- (i) Determine precise localization, levels of expression and specificity of NOX isoforms in the CNS;
- (ii) Evaluate the role of NOX during ND progression in animal models of ND and patients;
- (iii) Identify and characterize small molecules targeting NOX as potential therapeutics for NDs;
- (iv) Develop reliable approaches to measure NOX activity in vivo; and
- (v) Identify the molecular pathways controlled by NOX during neuroinflammation;
- (vi) Address the correlation of NOX activity and disease progression and severity in prospective clinical trials (biomarkers).

Selected diseases with a strong neuroinflammatory contribution were selected to address the above-mentioned questions.

These included ND related to aggregated proteins, such as ALS, mesial temporal lobe epilepsy and autoimmune demyelination of the CNS (MS) and the peripheral nerves (Guillain-Barre or chronic inflammatory demyelinating polyneuropathy).

The NEURINOX consortium consisted of 13 entities, mostly composed of biomedical researchers active in the fields of ND and NOX, clinical centers and SMEs involved in the development of NOX therapeutics.

Figure 2 shows the organization of the NEURINOX consortium specifically dedicated to reach the above-mentioned objectives.

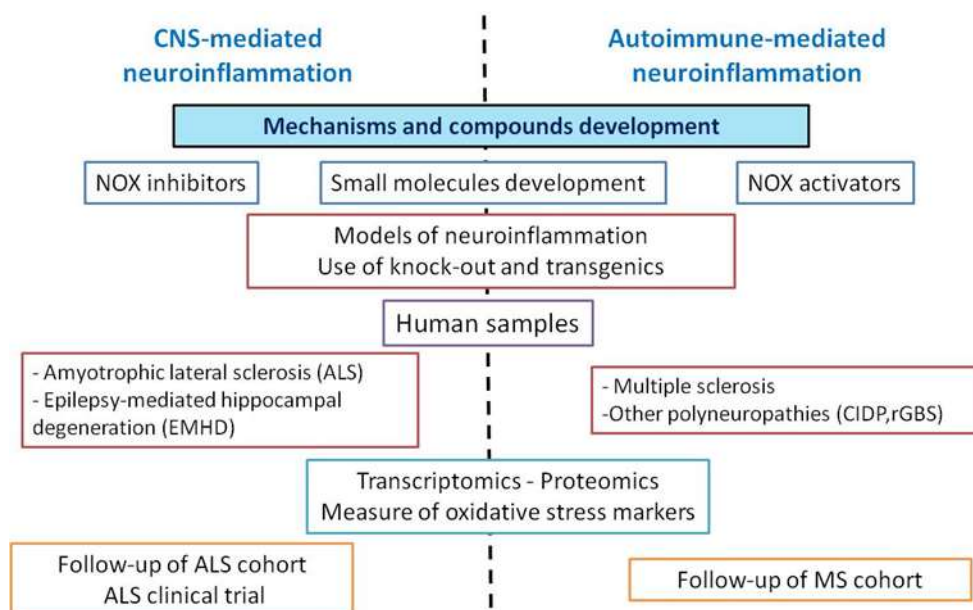


Figure 2: Schematic representation of the workflow of NEURINOX.

1.3 Description of the main S&T results/foregrounds

(i) NOX2 and NOX4 are the main CNS NOX isoforms. Combination of qPCR, immunostaining and available RNAseq databases indicate that under physiological conditions most NOX isoforms are expressed only to a negligible extent in the CNS.

NOX1, NOX3, NOX5, DUOX1 and DUOX2 are undetectable or at the limit of detection in the human and mouse CNS ([Jaquet V 2016](#)) ([Zhang Y, 2014](#)) Only NOX4 is detectable at basal conditions, and RNAseq data indicate that it is expressed mostly in the brain endothelium.

In terms of expression, immunostaining and RNAseq data show that the most prevalent NOX isoform in the CNS is NOX2. NOX2 is very specific for microglia although adult neural stem cells also express it.

In ND microglia is activated in the CNS of patients and mouse models of ND and NOX2 expression is enormously enhanced in several models of ALS ([Seredenina 2016](#)) and other ND ([Sorce 2014](#)). **Figure 3** summarizes cellular localization of NOX isoforms.

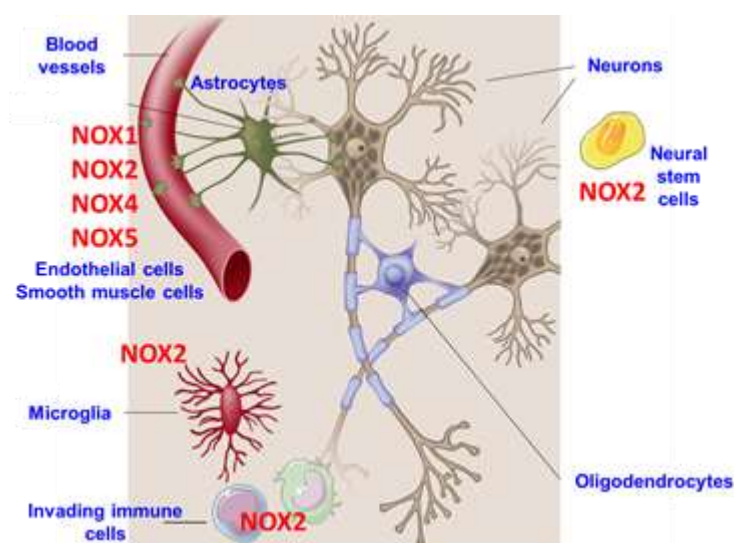


Figure 3: NOX expression in the CNS parenchyma. NOX2 is expressed in microglia and invading monocytes. NOX2 expression levels are strongly increased upon microglia activation. Neural stem cells of adult neurogenic regions also express NOX2. All other detected NOX isoforms (NOX1, NOX4 and NOX5) are expressed in brain vessels.

(ii) Proof of concept animal models using NOX knockout mice in ND models. Two models of ND known for protein aggregation and strong neuroinflammatory reaction were evaluated for NOX genetic inhibition: ALS and Creutzfeldt's Jakob disease (CJD).

We have shown that the expression of microglial NOX2 is strongly increased in both patients and animal models of ALS, specifically in affected regions of the spinal cord ([Seredenina 2016](#)). We crossed a well-characterized model of ALS (the SOD^{G93A} mice) with NOX2, NOX1 and NOX4 gene knockout mice.

However none of the genetic deletion of NOX isoforms did improve disease incubation time. In NOX2 deficient mice bred with ALS mice, neither microgliosis, nor astrogliosis nor motoneuron survival were changed ([Seredenina 2016](#)).

These results thus question initial reports ([Marden 2007](#)), showing a prolonged lifespan in ALS models related to NOX2 and NOX1 deficiency. Creutzfeldt Jakob disease (CJD) is an incurable ND characterized at the neuropathological level by a massive neuronal loss conferring a spongiform aspect of the brain and a neuroinflammatory environment showing increased microglial NOX2 expression ([Sorce 2014](#)).

A NOX2-deficient CJD mouse model showed a transient improvement of motor function and decreased vacuoles formation and brain oxidant levels, but microgliosis was not affected significant survival improvement and improvement of vacuoles formation. Similarly, in another model of aggregation-induced ND, it was shown that NOX2 greatly contributes to neurotoxicity ([Sonati 2013](#)).

(iii) NOX inhibitors. Most reported NOX inhibitors are unspecific ([Heumuller 2008](#); [Hirano 2015](#)): they act as oxidant scavengers, inhibit upstream activators of NOX ([Gatto 2013](#)) or interfere with the assays used to measure NOX activity ([Dikalov 2014](#)).

Thus, when testing potential small molecules NOX inhibitors, it is essential to use methods measuring both consumption of the substrates (NADPH, O₂) and the products (O₂⁻, H₂O₂) of the reaction catalyzed by NOX ([Jaquet 2009](#)) to exclude false positives including those resulting from interference

with oxidant-measuring systems (e.g. amplex red) Using this approach, we identified that a class of anti-psychotic agents have NOX inhibitory activity. Indeed a subset of *N*-substituted phenothiazines - but not non-substituted phenothiazines – are *bone fide* NOX inhibitors ([Seredenina 2015](#)).

These molecules are brain permeant and are already used as human therapeutics and can potentially be repurposed in ND. We also characterized a novel NOX2 inhibitor GSK2795039 and showed that it is competitive for the NADPH binding site of NOX2. GSK2795039 is orally available, non-toxic, CNS-permeable and inhibits NOX2 activity in vivo ([Hirano 2015](#)). Administration of thioridazine and perphenazine to SOD^{G93A} mice did not improve survival, although they showed some benefits in secondary read-outs, such brain oxidant levels, motor function for thioridazine and weight loss for perphenazine ([Seredenina 2015](#)).

(iv) NOX activators. In the context of autoimmune diseases NOX2 activity is anti-inflammatory. This unexpected fact was originally discovered in a genetic study performed to identify polymorphic loci controlling autoimmune chronic inflammatory diseases, using models for rheumatoid arthritis and multiple sclerosis ([Olofsson 2007](#)). This led to the identification of a polymorphism in the coding sequence of Ncf1 (neutrophil cytosolic factor 1), the gene coding for p47^{phox}, a subunit essential for the generation of O₂^{•-} by NOX2 ([Olofsson 2003](#), [Hultqvist 2011](#)). NOX2-derived oxidants generated by macrophages and other antigen presenting cells regulate activation of autoreactive T cells ([Gelderman 2007](#))

Since then, a protective role of NOX2-derived oxidants has been reproduced in several different models of autoimmune disorders, including models for MS and Guillain Barré ([Becanovic 2006](#)), ([Hultqvist 2004](#)). The NOX2-dependent anti-inflammatory effect is mediated by several different pathways: (i) downregulation of autoreactive T cells during antigen presentation ([Gelderman 2007](#)); (ii) autocrine downregulation of inflammatory macrophages ([Holmdahl, 2016](#)), ([Khmaladze 2014](#)) (iii) downregulation of STAT1 mediated activation of the interferon pathway ([Kelkka 2014](#)), ([Madhwal 2014](#)); and (iv) promotion of a protective effect by neutrophil extracellular traps (NETS) formed by neutrophils ([Schauer, 2014](#))

Further studies aimed to identify both the chemical nature of the oxidants involved and the respective roles played by these potentially protective pathways in MS and peripheral neuropathies are needed. Indeed, it is still unclear at present if excess oxidants in the CNS are counteracting the protective effect of the peripheral inflammatory attack, thereby promoting neurodegeneration ([Schuh C 2014](#)). Thus although the NOX inhibitory approach appears valid to decrease neuroinflammation, NOX activation may have therapeutic benefit for autoimmune-mediated neurodegeneration, including MS and other peripheral demyelinating neuropathies.

The NEURINOX partner, Redoxis AB has developed and characterised novel molecules able to enhance NOX2 activity with the objective to treat CNS autoimmune diseases ([Hultqvist 2015](#)) ([Holmdahl 2004](#)), ([Wallner 2012](#)). Identified NOX agonists have anti-inflammatory properties and are able to decrease the pro-inflammatory role of TNF- α in the low nanomolar range and are currently being optimised for regulatory safety studies and selection of candidate drug for clinical evaluation in ND.

(v) Measurement of NOX activity in vivo. Measuring NOX activity in tissues is a challenging task. Hydroethidine or dihydroethidium ([Kalyanaraman 2010](#)) is the 'gold standard' for O₂^{•-} determination in biological systems ([Dikalov 2007](#)). 2-hydroxyethidium is a specific oxidative product of O₂^{•-}. 2-hydroxyethidium can be measured by a combination of chromatographic separation of ethidium and 2-hydroxyethidium coupled with fluorescent or mass spectrometry. Using the LC-MS/MS approach, we showed that following hydroethidine into the spinal cord revealed that thioridazine decreased O₂^{•-} in the spinal cord of SOD1^{G93A} mice *in vivo* ([Seredenina 2016](#))

(vi) NOX and oxidative biomarkers in NDs. A large part of NEURINOX was dedicated to ND patients with the objective to evaluate NOX activity and oxidized biomarkers during ND progression and severity. In a prospective clinical study, NOX2 activity from peripheral neutrophils and monocytes was directly measured in fresh whole blood of a cohort of 83 ALS patients, and age- and gender-matched healthy controls. Upon addition of a specific activator, no difference was observed between patients

and healthy controls, however, inside the ALS group, low NOX2 activity in leukocytes was significantly associated with a longer survival ([Marrali, 2014](#)).

This represents an important finding in the field of ALS because this may be a prognosis biomarker of the severity of ALS.

More importantly, such a measure could be implemented as surrogate biomarker to address the benefit of a drug in clinical studies. A similar approach was used with patients affected by chronic inflammatory demyelinating polyneuropathy (CIDP) a neurological disorder characterized by damaged myelin sheath of the peripheral nerves. Intravenous immunoglobulin (IVIg) therapy is used as a first-line therapy and usually provides substantial benefit to patients. A prospective clinical study enrolled 30 CIDP patients treated with IVIg and 30 control subjects for whom NOX2 activity was measured in neutrophils and monocytes from freshly collected blood. At diagnosis NOX2 activity was significantly increased in CIDP patients compared to controls. However, following IVIg therapy, NOX2 activity was even more increased compared to basal levels ([Marrali, 2016](#)). The exact cause of this observation is unclear, but the results are consistent with therapeutic improvement in autoimmune demyelination being associated with enhanced NOX2. Because of the simplicity and robustness of this assay, it should be included systematically in clinical settings for ND.

This would potentially provide key information on inclusion criteria and the response to a drug. Another approach was used to determine levels of F₂-isoprostanes by LC-MS/MS in cerebrospinal fluid and plasma of patients with progressive MS. Compared with controls, plasma concentrations of F₂-isoprostanes and prostaglandin F₂ (PGF₂) were decreased with increasing disability score ([Lam 2016](#)). This was in contrast to the situation in cerebrospinal fluid, where the concentrations of PGF₂, but not F₂-isoprostanes, were significantly higher in patients with progressive disease than controls. Cerebrospinal fluid PGF₂ was reduced with natalizumab and methylprednisolone treatment, suggesting that PGF₂ levels in the CSF represents reliable surrogate biomarkers for evaluation of the efficacy of a drug. These results suggest that MS progression is associated with low rather than high systemic oxidative activity, and that this may play a role in immune dysregulation with central nervous system inflammation accompanied by increased local cyclooxygenase-dependent lipid oxidation.

Altogether the results obtained by the NEURINOX consortium led to numerous findings related to ND, oxidative stress in mouse models of ND and patients affected by ND. These findings are documented in 78 papers and 3 patents. More specifically, NEURINOX clarified NOX localization in the CNS, identified novel small molecule NOX inhibitors/activators and state-of-the-art methods to measure O₂^{•-} *in vivo*, showed a strong association between NOX2 and disease progression in ND, and indicated that NOX2 expression and activity parallels microgliosis and neuroinflammation in ND. However, inhibition of NOX provided only limited beneficial effects in ND. NOX2 up-regulation is certainly a common feature of ND and a sign of a neuroinflammatory response, but NOX2 inhibition is not a disease-modifying treatment. NOX2 upregulation, increased oxidant generation, microgliosis and neuroinflammation are all associated factors of ND, and rather represent a consequence of the neurodegenerative process. One of the key findings of our studies is the correlation between NOX2 activity and ALS progression, which makes NOX2 a promising biomarker for future evaluation of therapies for ND.

To advance the development of efficient drugs for ND-targeting redox systems it will be essential to use and propagate reliable molecular probes to identify:

- (i) The pattern of expression (RNAseq, antibodies);
- (ii) Inhibit specific oxidant-generating systems (small molecules, CRISPR-CAS);
- (iii) Localize and quantify specific reactive oxygen species *in vivo* to understand how and which therapeutics should be used and,
- (iv) Identify the impact of oxidative modifications of target proteins on cell functioning by redox proteomics. Understanding the kinetics of oxidant formation and metabolism, the relative role of various oxidant-generating systems and their inter-dependence in the fine redox regulation will pave the way for long awaited therapeutics targeting oxidative stress in CNS disorders.

1.4 Potential impact

1.4.1 Conclusions to date regarding the role of NOX in ND and impact on future research

The results obtained by the NEURINOX consortium clarified NOX localization in the CNS, identified novel small molecule NOX inhibitors/activators and state-of-the-art methods to measure $O_2^{\cdot-}$ *in vivo*, showed a strong association between NOX2 and disease progression in ND, and indicated that NOX2 expression and activity parallels microgliosis and neuroinflammation in ND. Importantly, however, inhibition of NOX provided at best only limited beneficial effects in ND. NOX2 up-regulation is indeed a common feature of ND and a sign of a neuroinflammatory response, but NOX2 inhibition is not a disease-modifying treatment. One of the key findings of our studies shows that a correlation between NOX2 activity and disease progression or response to treatments in patients is measurable in the blood, which makes NOX2 a promising biomarker for future evaluation of therapies for ND.

1.4.2 Description of the expected final results, potential impact and use

The NEURINOX consortium is the first concerted effort to gain a comprehensive view of the implication of ROS-generating NOX enzymes in neuroinflammation. NEURINOX contributes to better understand brain dysfunction and more particularly the link between neuroinflammation NOX enzymes and aims at identifying new therapeutic targets for neurodegeneration. A successful demonstration of the benefits of NOX modulating drugs in ALS, MS and CIPD animal models, and in ALS pre-clinical trials can validate novel high potential therapeutic targets for ALS and also other neurodegenerative diseases. Final expected results are the following:

- A better understanding of common mechanisms of brain diseases, and in particular oxidative stress-mediated neurodegeneration and the link between the different NOX isoforms and neuroinflammation and how their activities control the neuroinflammatory process in neurodegenerative disease.
- Novel oxidation biomarkers, molecular pathways, genes and SNPs correlating with NOX activity and ND for neurodegenerative and autoimmune-mediated neuroinflammation.
- Small molecules (NOX inhibitors and NOX activators), validated by NEURINOX partners in animal models for efficacy and mechanism of action (MOA).
- Validation of NOX as viable targets for the development of therapeutics for selected neurodegenerative diseases.

With these results, the NEURINOX research have the following impacts:

- **Impact on better understanding of brain function and redox regulation.** The NEURINOX results allow a better understanding of the role of NOX in ND, including ALS, MS and MTLN-HS. It also helps identifying more general redox mechanisms involved in brain function and ND
- **Impact on better management of neuroinflammatory and subsequent neurodegenerative diseases.** Costs of ND to society are huge and a breakthrough in the treatment of ND will allow great economic gains. By exploring a new therapeutic approach in ALS and NOX activity as new biomarkers of ND progression, NEURINOX contributes to the improvement in clinical management of ND and hence to a reduction of health care costs.
- **Impact on public health.** NOX-mediated therapeutics may be used for slowing progression of neurodegenerative diseases, which are so far untreatable
- **Impact on ND research and for structuring European research efforts.** NEURINOX brings together international experts in neuroinflammation and related areas and also seeks collaboration with other European initiatives in the area of ND research, thus contributing to structuring European research efforts..
- **Impact on competitiveness of European industry.** NEURINOX aims at creating new knowledge and translating it into novel therapeutic targets through the involvement of a number of SMEs who

are well positioned to derive new therapeutic products from the project results. Through industrial collaboration, the proposed work is increasing the competitiveness and is boosting the innovative capacity of European health-related enterprises, which is a global priority of the FP7 HEALTH programme.

1.4.3 Dissemination and Exploitation Activities

To deal with the questions related to dissemination of results, exploitation and IPR a specific work package (WP9) was setup. The aim of the dissemination actions was to reach the identified target audience and to transfer correct and incisive information about the project activities and achievements. It was also the objective of the consortium to communicate around the collaborative actions made feasible thanks to the support of the European Commission.

The consortium has therefore designed a plan for disseminating knowledge with the objectives:

- To raise **public** participation and awareness of the progress made within NEURINOX
- To enhance exchanges with **scientific world**
- To prepare **exploitation** of results, create market opportunities
- To **spread the knowledge** gained beyond the consortium

The dissemination activities have been organised in four large parts by target group: Dissemination towards:

- the public
- the scientific world
- the LifeSciHealth Programme
- the industry

For each of the target groups the aim, content, specific target, main message, detailed activities and timing of activities are described in the tables below.

Public Website

Target audience	<ul style="list-style-type: none"> • The citizen/consumer • Scientists of the live sciences / health sector • Audience interested in collaborative research projects • The industry
Communication channel	<ul style="list-style-type: none"> • The web
Objective	<ul style="list-style-type: none"> • The website presents the NEURINOX project and partners, and provides information about NOX and its role in neuroinflammation and ND in four dedicated sections addressing the individual target groups: the general public and patients, researchers and clinicians, pharmaceutical industry, and people in education: http://www.NEURINOX.eu/ • It will inform the target audience about the research and results of NEURINOX
Expected impact	<ul style="list-style-type: none"> • Contribute to reach general audience and to give easily understandable information about research on the role of NOX enzymes in neuroinflammation and evaluate their potential as drug targets for the treatment of neurodegenerative diseases. Contribute to also provide scientific details on advancement and results obtained by the NEURINOX project to

	researchers and professionals of the health sector. In the same time, increase awareness of people on the benefit of European research programmes for the citizen and the need to support the effort invested in research in the EU		
Content	<i>Section</i>	<i>Sub-section</i>	<i>Content</i>
	Home Page	N/A	Introduction of the aim of the project, its funding and composition of the consortium
	About NEURINOX	Overall concept and strategy	Details on the project objectives, innovation, work organisation and expected results
		Scientific approach	Explanation on the project state-of-the-art and the scientific research.
		Consortium partners	Short presentation of each partner: (very) short presentation of the organisation, main contribution to the project, link to the corporate website
	Patient information	N/A/	Presentation of different neurodegenerative diseases: Epilepsy, ALS, MS
	Educational Material	N/A	Power Point presentations on different methods and technologies used by NEURINOX partners and presentations made by invited speakers and last year dissemination activities : NEURINOX Graphic novel and the conference given by the scientist to high school students.
	Opportunities for Industry partnerships	Form to complete	To industries interested in discussing opportunities for partnerships with the NEURINOX partners
	Publications	Publications Related publications	Listing related publications issued prior and within the duration of NEURINOX
	News & Events	N/A	List of News
	Project Documents	Project flyer and yearly publishable summary	Keep the target audience aware of the research and results of NEURINOX
	Publications	Publications and public material (press release, logo, leaflet, ...) produced by MetaFight	Managed by ARTTIC with inputs from ALL

	NEURINOX Symposium	Divided in 3 subsections corresponding to the structure of the event	PowerPoint presentation dedicated to Day1: patients' associations/public/journalists Day2: scientific and industry communities Day 3: Videos produced by the students selected to participate in the event.
Date(s)	First version: on-line in M9. Updated continuously within the project duration for sections New & Events and Publications, as needed for the other sections		

Preparation of project presentation material

Target audience	All
Means	<ul style="list-style-type: none"> • Project logo • Standard PPT lay-out • Standard NEURINOX presentation (PPT)
Objective	<ul style="list-style-type: none"> • Provide basic presentation material to support information dissemination actions
Expected impact	<ul style="list-style-type: none"> • Consistent and professional image of the project
Content	<ul style="list-style-type: none"> • Standard project presentation (PPT).

Media relations

Target audience	<ul style="list-style-type: none"> • The citizen/patients • Scientists • Health professionals • Stakeholders
Means	<ul style="list-style-type: none"> • Project flyer, press releases
Objective	<ul style="list-style-type: none"> • Inform the target audience about the research and results of NEURINOX • Inform them about the objectives and scope of publicly funded European research programmes
Expected impact	<ul style="list-style-type: none"> • Contribute to reach general audience and to give easily understandable information about research on the role of NOX enzymes in neuroinflammation and evaluate their potential as drug targets for the treatment of neurodegenerative diseases. • Contribute to also provide scientific details on advancement and results obtained by the NEURINOX project to researchers and professionals of the health sector. In the same time, increase awareness of people on the benefit of European research programmes for the citizen and the need to support the effort invested in research in the EU
Content	<ul style="list-style-type: none"> • Summary information on NEURINOX objectives and/or achievements, described in a commonly understandable language
Date(s)	<ul style="list-style-type: none"> • First press release on the occasion of the International Symposium • Next press release issued with the project graphic novel

Leaflet

Target audience	<ul style="list-style-type: none"> • Scientists • Stakeholders from the health sector
Communication channels	<ul style="list-style-type: none"> • Conferences and workshops • Face to face meetings with scientists and other persons interested in NEURINOX
Objective	<ul style="list-style-type: none"> • Make them aware about NEURINOX and research in the field of NOX
Expected impact	<ul style="list-style-type: none"> • Interest from other research groups and opportunities for the development of further collaborations
Content	<ul style="list-style-type: none"> • Overview on NEURINOX objectives, expected results, project partners

Scientific publications

Target audience	<ul style="list-style-type: none"> • Scientists
Communication channels	<ul style="list-style-type: none"> • Scientific journals
Objective	<ul style="list-style-type: none"> • Inform about findings resulting from NEURINOX project research
Expected impact	<ul style="list-style-type: none"> • Visibility of excellence of research work carried out by partners involved in NEURINOX
Content	<ul style="list-style-type: none"> • Results from research carried out in NEURINOX

Networking with other RTD projects

Target audience	<ul style="list-style-type: none"> • Experts from industry and research • Health programme community focused on NOX research
Projects/events	<ul style="list-style-type: none"> • Workshops organised by the Health programme • Collaboration with EU-ROS: The European Network on Oxidative Stress and Redox Biology Research, a COST action dedicated to redox mechanisms in health and disease (http://eu-ros.eu/)
Objective	<ul style="list-style-type: none"> • Attract interest from experts in the NEURINOX approach to generate additional research activity on NOX • Create synergies between projects working in related research fields and opportunities for exchanging knowledge
Expected impact	<ul style="list-style-type: none"> • Additional input/resources for NEURINOX research • Strengthened collaboration with other research groups
Content	<ul style="list-style-type: none"> • Objectives and achievements of NEURINOX

High School Students outreach program "Biosciences: Open your mind -Change your world"

Target audience	<ul style="list-style-type: none"> • High-school students.
Objective	<ul style="list-style-type: none"> • Provide Students an overview of neurodegenerative diseases and related research approaches, real-life experience of innovative research conduction, and career orientation advice for biosciences to the attendees.
Expected impact	<ul style="list-style-type: none"> • Be a source of inspiration that triggers students' creative curiosity and help them broaden their perspective.
Content	<ul style="list-style-type: none"> • Scientific lectures, discussions, laboratory visits, demonstrations of

	experiments, use of microscopes/stereoscopes, collaboration games, motivational talks and researchers' life stories.
Date(s)	• 2014/2015/2016

OMICS Technologies Summer schools

Target audience	• Graduate students and post-doctoral fellows
Objective	• Introduce the students and young research fellows to Omics technologies
Expected impact	<ul style="list-style-type: none"> • Introduce the experimental processes behind key Omics technologies (Genomics, transcriptomics, proteomics....) • Familiarize them with the broad range of Omics applications in the basic and clinical research, as well as the clinical practise settings • Provide ideas for experimental approaches that could be employed in the attendees' future projects • Experience how the actual processes are conducted through lab visits • Network with scientists and international students
Content	• A mix of keynote lectures from experts in the field, laboratory visits and hands-on training in Omics bioinformatical data analysis
Date(s)	• 2014/2015/2016

Summer school

Target audience	• PhD Students and post-doctoral researchers working in the NEURINOX Project, members of the international academic and industrial community
Objective	• Exchange views and knowledge in the context of the NEURINOX project.
Expected impact	<ul style="list-style-type: none"> • The interactions with the project partners • The feedback from other experts • The ability to exchange ideas for future experiments • The networking with other members of the consortium
Content	<ul style="list-style-type: none"> • 10 minute oral presentation from students and young researchers to introduce themselves, their research focus and the poster they would present during the event • Keynote lectures from NEURINOX senior scientists and invited speakers on NOX-related scientific topics, as well as career orientation issues.
Date(s)	• 15-17 September 2014

Winter School

Target audience	• PH.D and young scientists
Objective	• Create synergies between labs on exchanging knowledge
Expected impact	<ul style="list-style-type: none"> • Young researchers to meet with Senior experts from NEURINOX and senior invited PIs to have fruitful discussions and inputs related to NOX • Strengthened collaboration between research groups
Content	• Opportunity to discuss the already collected data on the role of NOX2/NOX4 in MTLE epileptogenesis.

Date(s)	• 16-17 March 2015
---------	--------------------

International Symposium

Target audience	• Public, patients associations, industry community and peers, journalists and policy markers
Objective	<ul style="list-style-type: none"> • provide visibility to the public, industry community and peers as well as to the scientific community on the work progress made within NEURINOX as well as the work plan till the end of the project • bring forward to the EC, scientific and industry communities the need for future research/funding • follow-up with the stakeholders interested in the NEURINOX research till the end of the project
Expected impact	<ul style="list-style-type: none"> • Inform the target audience about the research and results of NEURINOX • Attract interest from experts in the NEURINOX approach to generate additional research activity on NOX
Content	• Objectives and intermediate achievements of NEURINOX
Date(s)	• 23-24 September 2015

Graphic Novel

Target audience	• Adults and adolescents, patients, families of patients and the general public.
Objective	• raise awareness about epilepsy, multiple sclerosis, and what issues scientific research is trying to address.
Expected impact	• Explain to the general public different neuroinflammation diseases
Content	<ul style="list-style-type: none"> • It is a story about love and science, aiming to raise awareness about epilepsy, multiple sclerosis, and what issues scientific research is trying to address. • At the end of the story, the scientist is giving a lecture to high school students on different neuroinflammation diseases. The slides in layman language addressed to teachers and science communicators for high school students have also been posted on the education section of the public website.
Date(s)	• Sept 2016 for the English version, then in French version

Position Paper

Target audience	• Policy makers, financing organisations
Objective	• To reach committees of the European-Commission working for the European-Commission's draft of the Work Programme 2018-20.
Expected impact	• To be able to submit proposals in line with NEURINOX achievements and the need of additional research on neuroinflammation
Content	• overview of what has been achieved in NEURINOX and fields where continued research effort is needed to bring the NEURINOX findings closer to clinical application.
Date(s)	• December 2016

Bioinformatics platform

Target audience	• Scientific community
-----------------	------------------------

Objective	<ul style="list-style-type: none"> Website documenting all large scale data generated during the NEURINOX project (RNAseq, microarrays and proteomics)
Expected impact	<ul style="list-style-type: none"> Open-access to this platform will be provided in conjunction with the publication of the data. It will serve as a sustainable result of NEURINOX in the future.
Content	<ul style="list-style-type: none"> RNAseq, microarrays and proteomics
Date(s)	<ul style="list-style-type: none"> As soon as the data is published

Review article

Target audience	<ul style="list-style-type: none"> Scientific community
Objective	<ul style="list-style-type: none"> The results obtained in the context of the Neurinox consortium have been summarized in a review article entitled: NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: what is the evidence?" which is being submitted as invited contribution to the journal Free Radical Biology and Medicine.
Expected impact	<ul style="list-style-type: none"> In addition to the major conclusions derived from the last 5 years on the role of NOX in the CNS, the review suggests new directions for future investigations in this field of research.
Content	<ul style="list-style-type: none"> major conclusions derived from the last 5 years on the role of NOX in the CNS, the review suggests new directions for future investigations in this field of research.
Date(s)	<ul style="list-style-type: none"> April 2017

1.4.4 Exploitation of NEURINOX results

The WP9 leader, in collaboration with the Executive Board, has supported partners in their respective IP procedures, making sure that the access of NEURINOX results etc. are dealt with on a fair and viable basis for all. This task has included patent searches, filing of patent (or other IPR) applications, etc.

The research activities undertaken under the umbrella of the project have generated results with commercial potentials. This has raised the issues of intellectual property rights (IPRs), of protection of the property rights (confidential IP as well as patenting).

The NEURINOX consortium has been composed of research groups and clinical institutions, Universities and SME with extensive experience in NADPH oxidases (NOX) research and neurodegenerative diseases. They have exploited the results of the project in various ways.

The Research Centres and Universities have been mostly benefited from the advance in knowledge which have strengthen their position as leading research institutions in Europe and brought new opportunities for future partnerships. Concrete plans for exploitation covered mainly publications in peer-reviewed international journals and filing of patent applications. The general principles for IPR ownership and IPR protection have been established in the Consortium Agreement.

The NEURINOX results are used in three main ways by individual partners:

- Continuous research
- Dissemination by public presentations and publication
- Commercial development by confidential IPR and exploitation of patents

The following table highlights results and the use to be made by academic and research partners:

Partner	Results	Main use
UNIGE	Better understanding of the exact role of NOX in ALS and CNS pathologies	Focus on alternative sources of oxidative stress in neuropathological diseases.
	Optimal pharmacological tool to address the NOX inhibitory approach in ND	Allow a specifically timed inhibition of NOX2 and better address the potential of NOX2 inhibition in disease.
UT	In a prospective clinical study, we verified that NOX2 activity in leukocytes is significantly associated with survival in ALS patients as shown in our publication: Marrali, G.; Casale, F.; Salamone, P.; Fuda, G.; Caorsi, C.; Amoroso, A.; Brunetti, M.; Restagno, G.; Barberis, M.; Bertuzzo, D.; Canosa, A.; Moglia, C.; Calvo, A.; Chio, A. NADPH oxidase (NOX2) activity is a modifier of survival in ALS. <i>J Neurol</i> 261:2178-2183; 2014.	A proper modulation of NOX2 activity might hold therapeutic potential for ALS, suggesting further clinical researches on NOX role.
BRFAA/UGA	Redox proteomics has led to the Identification of potential novel targets of redox pathways.	Evaluate redox pathways in health and disease.
VCCRI	<p><u>Publications:</u></p> <ol style="list-style-type: none"> 1. Maghzal GJ et al. Detection of reactive oxygen species derived from the family of NOX NADPH oxidases. <i>Free Radic Biol Med</i> 2012;53:1903-1918 2. Maghzal GJ et al. Assessment of myeloperoxidase activity by the conversion of hydroethidine to 2-chloroethidium. <i>J Biol Chem</i> 2014;289:5580-5595 3. Maghzal GJ et al. Tryptophan catabolism is unaffected in chronic granulomatous disease. <i>Nature</i> 2014;514:E16-E17 4. Seredenina T et al. Evaluation of NADPH oxidases as drug targets in a mouse model of familial amyotrophic lateral sclerosis. <i>Free Radic Biol Med</i> 2016;97:95-108 5. Lam M et al. Absence of systemic oxidative stress and increased CSF prostaglandin F_{2α} in progressive MS. <i>Neurology Neuroimmunology Neuroinflammation</i> 2016;30;3(4):e256 6. Talib J et al. Detailed protocol to assess <i>in vivo</i> and <i>ex vivo</i> myeloperoxidase activity in mouse models of vascular inflammation and disease using hydroethidine. <i>Free Radic Biol Med</i> 2016;97:124-135 7. Sorce S et al. NADPH oxidases as drug targets and biomarkers in neurodegenerative diseases: what is the evidence? <i>Free Radic Biol Med</i> 2017; under revision. <p><u>Improvement of capabilities:</u> New methods to detect and quantify reactive oxygen species <i>in vivo</i>.</p> <p><u>Obtaining additional funds:</u> Stocker R. Australian Research Council Discovery</p>	Further research on the detection of reactive oxygen species.

	Project Grant 170101453: Novel methods to quantify multiple reactive species in biological systems. 2017-2019	
KI	<p>Publications :</p> <ol style="list-style-type: none"> 1. Rikard Holmdahl et al. A Reduction in Intracellular Reactive Oxygen Species Due to a Mutation in NCF4 Promotes Autoimmune Arthritis in Mice, Antioxid Redox Signal. 2016 Dec 20;25(18):983-996. Epub 2016 Jul 8. 2. Tuncel J et al. Animal Models of Rheumatoid Arthritis (I): Pristane-Induced Arthritis in the Rat. PLoS One. 2016 May 26;11(5):e0155936. doi: 10.1371/journal.pone.0155936. eCollection 2016. 3. Rikard Holmdahl et al Ncf1 polymorphism reveals oxidative regulation of autoimmune chronic inflammation. Immunol Rev. 2016 Jan;269(1):228-47. doi: 10.1111/imr.12378. 4. Sareila O et al. Direct Comparison of a Natural Loss-Of-Function Single Nucleotide Polymorphism with a Targeted Deletion in the Ncf1 Gene Reveals Different Phenotypes, PLoS One. 2015 Nov 3;10(11):e0141974. doi: 10.1371/journal.pone.0141974. eCollection 2015. 	Promote research on using NOX2 and related pathways as new targets for treatment of chronic inflammatory diseases and cancer.
All partners	<p>A bio-informatics web platform has been set up, documenting all large scale data generated during the NEURINOX project (RNAseq, microarrays and proteomics)</p> <p>It will service as a sustainable result of NEURINOX in the future.</p> <p>neurinox.vital-it.ch</p>	The platform addressed to the scientific community will be open-access in April 2017 in conjunction with the publication of the data.

The following table shows here the results and the use to be made by SME partners:

Partner	Project results	Timeframe for (commercial) use/ Target market
GKT	Novel NOX inhibitors targeting CNS pathologies. We have successfully identified and developed a potent and highly selective NOX1 inhibitor. This has led to a patent filing (PCT/IB2015/059659).	<p>Timeframe for (commercial) use 8-10 years after phase I completion, i.e. 2020</p> <p>Target market Neurodegenerative diseases, in particular Parkinson's disease.,</p>
	Further work needs to be done to improve brain penetration of the compounds. This could lead to new patents.	<p>Timeframe for (commercial) use 3-5 years after start of the project, i.e. 2015-2017</p> <p>Target market Potential outlicensing to large Pharmaceutical companies due to expected</p>

Partner	Project results	Timeframe for (commercial) use/ Target market
		long and complex clinical trials
RDX	<p>Have during the project investigated the effect of NOX2 activating small molecules on ND with good results. Due to species differences we had to do another round of chemistry to further optimize substances, which has left us with a delay in approaching the market (predicted 2018).</p> <p>Publication: Hultqvist, M.; Olofsson, P.; Wallner, F. K.; Holmdahl, R. Pharmacological Potential of NOX2 Agonists in Inflammatory Conditions. <i>Antioxid Redox Signal</i> 23:446-459; 2015</p>	<p>Timeframe for (commercial) use Redoxis subsidiary ProNoxis with VC investments for late preclinical and clinical development in 2014-2016 Partners involved: RDX & KI Target market Target market of autoimmune inflammatory disorders, CIDP, GBS, MS, RA and SLE. Stage of development: Preclinical lead development Intellectual property rights: Combination of patent application (WO 2012 127214 A1) and confidential compositional matter description.</p>
	<p>RDX have during the project established, standardized and validated a battery of preclinical models for ND in both rats and mice. This has resulted in an expanded market and several completed partnerships with industrial and academic partners. The complex modes of rat and recently mouse models of GBS/CIDP caused delay and request for additional budget.</p>	<p>Timeframe for (commercial) use Redoxis increased capacity for drug validation and industrial partnership during the program Target market Improved capacity to validate internal drug compounds and possibility for industrial partnership and commissions for drug validation in animal models</p>
	<p>IPR strategy has been completed with advice from patent attorneys at Potter Clarkson LTd and Awapatent. The consensus IPR strategy is a combination of public patent applications and confidential compositional matter as well as protection by orphan drug designation.</p> <p>Google Patents; 2012 Wallner, K. F. Olofsson, J. P. Hultqvist, M. K.; Pelcman, B. H. Quinolinone derivatives for use in the treatment of an autoimmune disease and/or an inflammatory disease.</p>	<p>Target market Increased possibilities to raise VC funds for late preclinical development. Partners involved: RDX & KI Possible market applications: Biotech and pharmaceutical corporate venture Stage of development: Preclinical lead development.</p>
NEURIX	<p>The Neurinox project allowed NEURIX to develop a human in vitro screening system as planned. The system is now active in proof-of-concept collaborative project with the industry.</p>	<p>Timeframe for (commercial) use Within 1 year: as soon as results generated by NEURINOX are publically available. N.B.: Neurix intellectual property will only cover the neuroinflammation models Target market Departments within pharmaceutical companies that work on degenerative diseases</p>

1.5 NEURINOX partners and contact

NEURINOX Coordinator

- 1) Dr Vincent Jaquet
Department of Pathology and Immunology
Faculty of Medicine
University of Geneva
1, rue Michel-Servet
1211 Geneva 4, Switzerland
Phone: +41 22 379 4136
E-mail: Vincent.Jaquet@unige.ch
NEURINOX public website: <http://www.NEURINOX.eu>

Partners

- 2) University of Zürich (UZH)
Neuropathology Institute
Schmelzbergstrasse 12
8091 Zurich, Switzerland
<http://www.en.neuropathologie.usz.ch/>
- 3) GenKyoTex SA (GKT)
16, Chemin des Aulx
CH-1228 Plan - Les-Ouates – Geneva, Switzerland
<http://www.genkyotex.com>
- 4) Redoxis AB (RDX)
Medicon Village
Scheelevägen 2
223 81 Lund, Sweden
<http://www.redoxis.com/>
- 5) ARTTIC (ART)
Dominique Wasquel / Sara Skogsäter
58A rue du Dessous des Berges
75013 Paris, France
<http://www.arttic.eu>
- 6) University of Torino (UT)
Department of Neuroscience
University of Torino
Via Cherasco 15
10126 Torino, Italy
<http://www.unito.it/>
- 7) Karolinska Institute (KI), Sweden.
 - a) Section for Medical Inflammation Research (MIR)

Dept of Medical Biochemistry and Biophysics
b) Neuroimmunology Unit (NU)
Department of Clinical Neurosciences
Center for Molecular Medicine
<http://www.ki.se/>

- 8) Université Grenoble Alpes (UGA) previously called Joseph Fourier University (UJF)
621 avenue Centrale
38400 Saint-Martin-d'Hères
<http://www.univ-grenoble-alpes.fr/en/>
- 9) SynapCell SAS (SYN)
Bâtiment Biopolis
5 avenue du Grand Sablon
38700 La Tronche, France
<http://www.synapcell.fr/>
- 10) Biomedical Research Foundation Academy of Athens (BRFAA)
4 Soranou Ephessiou Street,
Athens 115 27, Greece
<http://www.bioacademy.gr/>
- 11) University of Athens (UOA)
National and Kapodistrian University of Athens,
Clinical Genomics and Pharmacogenomics Unit,
4th Department of Internal Medicine,
Attikon Hospital, Medical School,
75 Mikras Asias
115-27 Athens, Greece
<http://en.uoa.gr/about-us.html>
- 12) University of Sydney (USYD) terminated in October 2012
Parramatta Road, 92-94
Camperdown NSW 2006, Australia
www.usyd.edu.au
- 13) NEURIX (NEURIX)
14 Chemin des Aulx
Plan les Ouates 1228
Switzerland
<http://www.neurix.ch/>
- 14) Victor Chang Cardiac Research Institute Limited (VCCRI)
School of Medical Sciences,
University of New South Wales, Australia.
405 Liverpool Street
Darlinghurst, 2010, Australia
www.victorchang.com.au