

**NINIVE**  
**Non Invasive**  
**Nanotransducer for In**  
**Vivo gene thErapy**

Proposal/Contract no.:  
**STRP 033378**



SIXTH FRAMEWORK PROGRAMME

Project acronym: **NINIVE**

Project full title: **Non Invasive Nanotransducer for *In Vivo* gene thErapy**

Proposal/Contract no.: **STRP 033378 NINIVE**

Instrument Type: **Specific Targeted Research or innovation Project**

Priority 3 – **NMP** (Nanotechnologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices)

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**Deliverable D8.1 “Medical & regulatory framework: applicability of NINIVE nanotransducer as intracortical non-invasive tool”**

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<b>Dissemination Level</b>		
<b>PU</b>	Public	<b>X</b>
<b>PP</b>	Restricted to other programme participants (including the Commission Services)	
<b>RE</b>	Restricted to a group specified by the consortium (including the Commission Services)	
<b>CO</b>	Confidential, only for members of the consortium (including the Commission Services)	

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## **1. Executive Summary**

*This report describes the medical & regulatory framework related to the applicability of the NINIVE nanotransducer as intracortical non-invasive tool.*

TA, p. 47

### Acronyms

ESGT: European Society of Gene therapy

TA: technical annex

NIH: National Institutes of Health

EMA: European Medicines Agency

## 2. Introduction

NINIVE project aims at developing a novel nonviral vector for gene delivery, able of a) gene transfection *in vivo* and on a large amount of cells, b) local and non invasive therapy, c) frequent and easy medication. In order to prove the project paradigms, *in vivo* validation of the NINIVE vectors will be performed. A specific neurological disorder will be treated to demonstrate the therapeutic potential of the designed vectors.

In agreement with the project TA:

1. NINIVE research activity will be focused on **basic research**, following a multidisciplinary approach and the integration of expertises in the fields of nanotechnologies, biotechnologies, communications technologies and neuroscience.
2. Animal trials will be performed to assess the efficiency, safety and selectivity of gene transfection via NINIVE vectors.
3. No clinical trials will be performed within the projects (36 months), in agreement to the NIH and ESGT recommendations.

NINIVE Consortium confirms that project research does not involve research:

1) on human beings, 2) on human biological samples, 3) on human embryos, 4) on human genetic information, 5) on other personal data, 6) on dual use, 7) involving developing countries (e.g. clinical trials, use of human and animal genetic resource), 8) aimed at human cloning for reproductive purposes, 9) intended to modify the genetic heritage of human beings which could make such changes heritable and 10) intended to create human embryos solely for the purpose of research or for the purpose of stem cell procurement.

The definition of Gene Therapy, provided by the ESGT in the Position paper on social, ethical and public awareness issues in gene therapy (.November 2002), is:

*Gene therapy is a technology by which genes or small DNA or RNA molecules are delivered to human cells, tissue or organs to correct a genetic defect, or to provide new therapeutic functions for the ultimate purpose of preventing or treating diseases.*

Having this in mind, we would like to underline the concept that:

Gene Therapy is not the use of genes for genetic manipulation of the human genome or its alteration creating new species, as NINIVE vectors aims to introduce genes into genome of somatic cells and we do not perform germ line modifications; Gene therapy is thus exclusively the use of recombinant genetic material (DNA, RNA) under different forms or pharmaceutical preparations as therapeutic agent.

### 3. Applicable regulatory framework related to biotechnologies

Concerning all the research activities related to biotechnologies, the NINIVE Consortium is conform to:

- The Charter of Fundamental Rights of the EU;
- Directive 98/44/EC of the European Parliament and of the Council of 6 July 1998 on the legal protection of biotechnological inventions.

Concerning the main potential risks arising from the abuse of gene therapy, we have identified:

1. *Creation of genetically modified organisms.* NINIVE does not support Gene Therapy technology for the creation of genetically modified organisms. NININE Consortium declares that Gene Therapy research and technology developed within the project will be not used for the creation of genetically modified organisms.
2. *Alteration of genome of a human being.* Gene Therapy technology to perform germ line cell modification is considered undesirable and unethical in the European Union, the United States and the vast majority of the economically and scientifically developed countries. As European project research involved in the development of gene transfer technology, NINIVE shares these ethical and social concerns and does not support it. NININE Consortium declares that the proposed research does not involve research activity aimed at human cloning for reproductive purpose.
3. *Patient Safety in clinical trial.* Not applicable, as NININE research does not involve clinical trials.

Having in mind risks n° 1 and 2, the Consortium declared to be conform to Directive 90/219/EEC of 23 April 1990 on the contained use of genetically modified microorganisms and Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

Having in mind risk n° 3, since every technology aims to develop products (therapeutic agents in the case of Gene Therapy technology), it is fundamental to take into account the safety issues related to the product use since the phases of research, design, development and preclinical study of this product. There are many useful notes for guidance on the quality and preclinical aspects of gene

transfer medicinal products. They will be taken adequately into account, as explained in the following.

The ethical implications of Gene Therapy do not substantially differ from those of any other form of experimental medicine, as long as the human genome is not altered in an inheritable way. In the framework of NINIVE project, Gene therapy will be practiced with the goal of curing patients, and possibly increasing their quality of life.

#### 4. Protection of Animals

In accordance with the Amsterdam protocol on animal protection and welfare, animal experiments will be replaced with alternatives wherever possible. Suffering by animals will be avoided or kept to a minimum. Species that are closest to human beings are not involved. Altering the genetic heritage of animals and cloning of animals are not performed.

The use of rat and mouse cavies is indispensable to achieve the goals of the project; these species are the furthest from the human being, which have a neural physiology and neural functions similar to that found in humans. In addition, the development of neural diseases models and their validation on rat/mouse cavies is a well-established and consolidated research area.

The NINIVE Consortium declares that any effort will be made in order **to reduce** the number of animals used.

All the experiments on animals will be carried out in CNR and CDDR-ULSOP laboratories. CNR experimental procedures have been approved by the Istituto Superiore di Sanità and authorized by Ministero della Sanità (Protocol #129/2000, released on Dec. 13, 2000).

At CDDR-ULSOP laboratory, any experimental procedure by involving animals has been described and authorised by the UK Home Office (Project License no.: PPL/70/5910) and approved by the University of London ethical committees in compliance with the UK Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. In addition, all procedures used have been approved and are controlled by the local Veterinary Committee.

The following list summarizes the actions planned for animal protection:

- Eventually experimental new protocols will be developed in full observance to the national and European regulations.
- Confirmation of specific approval from relevant ethics committees in the various Member states should be provided before research commences;
- Dignity of animals will be respect;
- Any potential misuse of the technology will be avoided;

- Main expected effects of the procedures on the affected animals have been provided, task by task (task 3.4, 4.5-7, 5.6-8, 7.2-11, “Main adverse effect for the animals”, section 9.7 “Description of the experiments on animals”,TA); additional details will be provided during the research.
- The specific number of animals to be used should be reported and justified (see section 9.7 “Description of the experiments on animals” of TA) ;
- The report on ethical and social issues will form part of the annual report of the project.
- An expert on “Animal Welfare”, Dr. Lachapelle, is in the GMEE (Group of Medical and Ethical Experts) which supervises the project.

Concerning all the research activities related to the use of animals, the NINIVE Consortium is conform to: CONCILIUM DIRECTIVE of 24 November 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 (86/609/EEC).

Additional details are given in 9.6 “Research on animals” and 9.7 “Description of the experiments on animals” of TA.

## 5. Clinical applications and regulatory issues

The NINIVE Consortium completely agrees with the **Guidelines from European Science Foundation “Forward Look on Nanomedicine 2005”** and **EMA’s regulatory issues surrounding nanomedicines**. On the following, some important concepts are underlined.

**The field of ‘Nanomedicine’** is the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body. It was perceived as embracing five main sub-disciplines that in many ways are overlapping and underpinned by the following common technical issues:

- Analytical Tools
- Nanoimaging
- Nanomaterials and Nanodevices
- Novel Therapeutics and Drug Delivery Systems
- Clinical, Regulatory and Toxicological Issues

**The aim of ‘Nanomedicine’** may be broadly defined as the comprehensive monitoring, control, construction, repair, defence and improvement of all human biological systems, working from the molecular level using engineered devices and nanostructures, ultimately to achieve medical benefit.

In this context, NINIVE project, which aims at functionalizing CNTs with DNA to produce a vector for Gene Therapy, completely fits the final goal of developing new therapeutics and drug delivery systems.

Europe has harmonised the regulations related to medicines across European countries by the activities of the European Medicines Agency (EMA). It is essential to develop appropriate regulatory requirements to facilitate the safe and swift introduction of future nanomedicines into clinical practice, and to influence basic, translational and clinical research in the field. General directions are:

- 1) disease-oriented focus for nanomedicine;
- 2) case-by-case approach for clinical and regulatory evaluation of nanomedicines;

3) highly prioritised communication and exchange of information among academia, industry and regulatory agencies with a multidisciplinary approach.

It is very important to test toxicological effects of new materials for clinical applications. General EMEA directions are:

- 1) Improved understanding of toxicological implications of nanomedicines in relation to materials properties and proposed use;
- 2) Thorough consideration of the potential environmental impact, manufacturing processes and ultimate clinical applications in toxicological investigations for nanomedicines;
- 3) risk-benefit assessment for both acute and long-term effects of nanomedicines with special consideration on the nature of target disease.
- 4) a shift from risk assessment to proactive risk management at the earliest stage of the discovery and development of new nanomedicines.

According to EMEA's regulatory issues surrounding nanomedicines, NINIVE project will also take into account specific non-clinical issues dealing with nanomedicines; as regards *in vitro* models the mechanisms of cellular uptake will be studied to determine the pharmacodynamics of cellular toxicity and the therapeutic potential of CNTs. Moreover, *in vivo* studies will be performed to test not only toxicity in rodent species, but also absorption, distribution, metabolism and elimination pharmacology, safety pharmacology, genotoxicity, developmental toxicity, irritation and sensitization studies, immunotoxicology and carcinogenicity.

## 6. Directive and ethical aspects of implants in human body

The history of implantable devices in clinical practise started in the 1960s with the development of the first heart pacemakers to replace the autonomic rhythm of the heart. The most recent examples of active implants for functional electrical stimulation are stimulators to treat pain in patients with tumours and trembling caused by Parkinson's disease. Today it is possible to implant artificial devices in the human body in order to restore physiological functions and these are the essential reasons why potential and actual implants in the human body have large and important ethical consequences. As for the European legal background, specific and close importance should be attached to the Charter of Fundamental Rights of the EU, which is currently Part II of the Treaty Establishing a Constitution for Europe. This sets out the general principles of dignity, freedom, equality, solidarity, citizenship and justice, as well as integrity and inviolability of the body, with particular regard to informed consent (ART 3) and personal data protection (ART 8). Data protection issues are developed in Directive 95/46 and 2002/58. The precautionary principle is expressly referred to Article 174 of the EC Treaty as well as, in detail, by the Commission's Communication (2000/01) of 2 February 2000.

Concerning NINIVE vector, the following EC Council Directive on medical device is applicable:

Directive 90/385 → For the purpose of this Directive, the following definitions shall apply:

a) "medical device" means any instruments, apparatus appliance, materials or other article, whether used alone or in combination, together with any accessories or software for its proper functioning, intended by the manufacturer to be used for human beings in the:

-diagnosis, prevision, monitoring, treatment or alleviation of disease or injury,

-investigation, replacement or modification of the anatomy or of a physiological process,

-control of conception,

and which does not achieve its principal intended action by pharmacological, chemical, immunological or metabolic means, but which may be assisted in its function by such means;

b) "active medical device" means any medical device relying for its functioning on a source of electrical energy or any source of power other than that directly generated by the human body or gravity;

c) “active implantable medical device” means any active medical device which is intended to be totally or partially introduced, surgically or medically, into the human body or by medical intervention into a natural orifice, and which is intended to remain after the procedure.

NINIVE vector can be thus considered an “active implantable medical device”. NINIVE Consortium will follow the ACTIVE IMPLANTABLES DIRECTIVE (90/385/EEC), ANNEX 1:

### ***I. General Requirements***

1. The devices must be designed and manufactured in such a way that, when implanted under the conditions and for the purposes laid down, its use does not compromise the clinical conditions or the safety of patients. They must not present any risk to the persons implanting them or, where applicable, to other persons.

2. The devices must achieve the performances intended by the manufacturer, be designed and manufactured in such a way that they are suitable for one or more of the functions referred to in Article 1 (2) (a) as specified by him.

3. The characteristics and performances referred to in sections 1 and 2 must not be adversely affected to such a degree that the clinical condition and safety of the patients or, as appropriate, of other persons are compromised during the lifetime of the device anticipated by the manufacturer, where the device is subjected to stresses which may occur during normal conditions of use.

4. The devices must be designed, manufactured and packed in such a way that their characteristics and performances are not adversely affected in the storage and transport conditions laid down by the manufacturer (temperature, humidity, etc.).

5. Any side effects or undesirable conditions must constitute acceptable risks when weighed against the intended performance.

### ***II. Requirements regarding design and construction***

6. The solutions adopted by the manufacturer for the design and constructions of the devices must comply with safety principles taking account of the generally acknowledged state of the art.

7. Implantable devices must be designed, manufactured and packed in a non-reusable pack according to appropriate procedures to ensure they are sterile when placed on the market and, in the storage and transport conditions stipulated by the manufacturer, remain so until the packaging is removed and they are implanted.

8. Devices must be designed and manufactured in such a way as to remove or minimize as far as possible:

- the risk of physical injury in connection with their physical, including dimensional, features,
- risks connected with the use of energy sources with particular reference, where electricity is used, to insulation, leakage currents and overheating of the devices,
- risks connected with reasonably foreseeable environmental conditions such as magnetic fields, external electrical influences, electrostatic discharge, pressure or variations in pressure and acceleration,
- risks connected with medical treatment, in particular those resulting from the use of defibrillators or high-frequency surgical equipment,
- risks connected with ionizing radiation from radioactive substances included in the device, in compliance with the protection requirements laid down in Directive 80/836/Euratom, as amended by Directives 84/467/Euratom and 84/466/Euratom,
- risks which may arise where maintenance and calibration are impossible, including:
  - excessive increase of leakage currents,
  - ageing of the materials used,
  - excess heat generated by the device,
  - decreased accuracy of any measuring or control mechanism.

9. The devices must be designed and manufactured in such a way as to guarantee the characteristics and performance referred to in I. 'General requirements', with particular attention being paid to:

- the choice of materials used, particularly as regards toxicity aspects,
- mutual compatibility between the materials used and biological tissues, cells and body fluids, account being taken of the anticipated use of the device,
- compatibility of the devices with the substances they are intended to administer,
- the quality of the connections, particularly in respect of safety,
- the reliability of the source of energy,

- if appropriate, that they are leakproof,
- proper functioning of the programming and control systems, including software.

10. Where a device incorporates, as an integral part, a substance which, when used separately, is likely to be considered to be a medicinal product as defined in Article 1 of Directive 65/65/EEC, and whose action in combination with the device may result in its bioavailability, the safety, quality and usefulness of the substance, account being taken of the purpose of the device, must be verified by analogy with the appropriate methods specified in Directive 75/318/EEC, as last amended by Directive 89/341/EEC.

11. The devices and, if appropriate, their component parts must be identified to allow any necessary measure to be taken following the discovery of a potential risk in connection with the devices and their component parts.

Concerning all the research activities related to the study of environmental impact of nano-medical products the NINIVE Consortium is conform to Directive 2004/27/EEC.

NINIVE is a device that we can considered an *Active therapeutic device* that used alone or in combination with other medical devices, to support, modify, replace or restore biological functions or structures with a view to treatment or alleviation of an illness, injury or handicap. For this definition NINIVE Consortium is conform to Directive 93/42/EEC following general requirements regarding design and construction about chemical, physical and biological properties declared in the Annex1.

NINIVE Consortium respects the Convention on Human Rights and Biomedicine of the Concilium of Europe (1997) and UNESCO'S Universal Declaration on the Human Genome and Human Rights (1997), in particular as regards respect for the dignity and integrity of individuals.