SIXTH FRAMEWORK PROGRAMME
HORIZONTAL RESEARCH ACTIVITIES INVOLVING SMEs

CO-OPERATIVE RESEARCH PROJECT

Project Number:	**513190**
Project Acronym:	**ION**
Project Title:	**"A sequential high throughput ion channel screening system for drug discovery in neurological and psychiatric disorders"**
Start date of project:	**December, 1st 2004**
Duration:	**24 Months**
Project coordinator name:	**Patricia Salvati**
Project coordinator organisation name:	**Newron Pharmaceuticals S.p.A.**

PUBLISHABLE FINAL ACTIVITY REPORT

Related to the period from month 1 to month 24

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Prepared By:

Dr. Patricia Salvati – ION Project Coordinator
E-mail: patricia.salvati@newron.com
Dr. Carla Caccia – ION Project Manager
E-mail: carla.caccia@newron.com

Newron Pharmaceuticals SpA
Via L. Ariosto, 21
20091 Bresso (MI) – Italy
Phone: +39 02 6103461
Fax: +39 02 61034635

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INTRODUCTION

This document is the Publishable Final Activity Report for the activities carried out by the ION Consortium during the twenty-four months of the project execution.

The Final Activity Report provides a summary description of project objectives, partners involved, work performed and results achieved.

The section 2 “Dissemination and Use” includes the publishable results of the Final Plan for Using and Disseminating the Knowledge.

It has been prepared by Mrs. Patricia Salvati, the ION Project Coordinator (Newron Pharmaceuticals Spa), and Mrs. Carla Caccia, the ION Project Manager (Newron Pharmaceuticals Spa), and discussed with all the ION partners who approved it.

In order to complete this report, the ION partners took advantage of the ION Communication and Cooperation System already in place to ensure remote cooperation among project partners.

This document represents the publishable activity report due to the Commission at the end of month twenty-four of the project execution, as foreseen in the contract.

1. PROJECT EXECUTION

Project title:

"**A Sequential High Throughput Ion Channel Screening System for Drug Discovery in Neurological and Psychiatric Disorders**".

Project acronym:

ION

Project logo:

Executive Summary:

ION is a co-operative research project financed by the European Commission through the Sixth EU Framework Programme for Research and Technological Development.

The signed research contract is of € 1.833.160, with a Commission contribution of € 1.275.340.

The duration of the project was 24 months.
The starting date was December, 1st 2004 and the project end was November, 30th 2006.

Project Background:

New medicines emerge from a long and difficult research and development process that often begins with a huge number of candidate chemicals being tested, or "screened", for useful biological effects.
The domain of interest of the ION project was the screening of candidate drugs in relation to their ability to affect, at the bio-molecular level, ion channels, which control the flow of ions across biological membranes.
Relationships among diseases and the behaviour of ion channels are found in a wide range of pathologies, including very common neurological and psychiatric disorders such as depression, Parkinson's disease, epilepsy, migraine and pain.

Project Objectives:

The main scope of the ION project was to develop technical systems capable to improve the speed and efficiency of testing of chemical compounds capable to act on ion channels, that might be used therapeutically. The ION project will optimize the performance of a sequence of screening experiments instead of focusing on the execution of a single experiment.

The innovative features of the ION systems addressed several points, ranging from the adaptation and the integration of current and emerging electrophysiology equipments, to the development of biological target libraries and intelligent software agents suitable to interpret and process the outcome of a screening experiment for a given biological target and its modulating chemicals.

The technological objectives of the ION project are of two different types:

- The first objective was the improvement of existing and emerging electrophysiology platforms by adapting them to the ION software and libraries. These improved experimental platforms are called ION Experimental Platforms (IONEP). In particular ION intends to develop an interfacing software agent to standardize the integration of electrophysiology equipments for the application of its approach to high throughput screening. The study, development and validation of the interfacing agent has been prototyped by adapting the MCS platform.

- The second objective was the selection and adaptation of an appropriate software technology (development environment) in order to design and implement the required software agents.

Finally, the ION project aimed at preparing a business plan in order to exploit the achieved results worldwide.

ION consortium members:

The project has been set up around four specialised European Small and Medium Enterprises (SMEs) based in Italy, Germany and Romania.

The necessary research and technological development (RTD) assistance was provided by five academic research institutes located in Italy, Poland and Hungary. RTD performers provides the knowledge and the research capabilities to address the selection and optimization of the cellular targets and to validate the outcomes of the system in relation to the results of in vivo models of the pathologies of interest. An other important contribution of the RTD partners is the design of a suite of innovative software agents for data analysis and experimental planning. The role of these agents is essential to achieve real high screening throughputs.

Here in the following a brief description of the ION consortium members.

SMEs:

The project coordination is assigned to an Italian SME: Newron Pharmaceuticals SpA (www.newron.com), which has appointed Mrs. Patricia Salvati and Mrs. Carla Caccia respectively as Project Coordinator and Project Manager.
Newron is a research and development company focused on novel ion channel based therapies for diseases of the Central Nervous System (CNS), particularly Parkinson's disease, epilepsy and pain. Newron uses screening technology in its drug development work performing the screening trials.

Multi Channel Systems MCS (www.multichannelsystems.com) is a German SME which offers online data acquisition and analysis of the PC-platform and produces high-end electronic devices for single and multichannel measurements. MCS has its core competence in the field of development and manufacturing of multi channel recording systems and automation.

Nikem Research (www.nikemresearch.com) is an Italian chemitechnology company spun-off from GSK offering a full range of medicinal, combinatorial and computational chemistry services, products and partnering possibilities for the pharmaceutical and biotechnological industries.

SC IT Romania (www.itr.ro) is a Romanian emerging software company in a candidate member state with important links with the Italian software industry (BBS Software) and it has solid experience in developing CRM applications, B2B projects, ASP applications, document management and Work Flow Automation solutions for international customers.

RTD Performers:

University of Debrecen (www.unideb.hu) is a Hungarian multi-disciplinary public research and educational body affiliated to the Ministry of Education. The major scientific activities are connected with the cellular and molecular basis of pathology of human diseases with a high socio-economic impact. The Department of Anatomy and Histology is under the supervision of Prof. M. Antal, a well recognized leader in the field of neuroanatomy and neurophysiology, in particular motor and sensory neural circuits in the spinal cord.

The Department of Preclinical and Clinical Pharmacology of the University of Florence (www.pharm.unifi.it) is an Italian large teaching, research and clinical institution offering PhD and master programmes in Pharmacology and Toxicology. The unit of Neuroscience is led by Prof. F. Moroni, widely recognized for his contributions to the fields of glutamate neurotransmission and excitotoxicity.

The "Institute of Pharmacology of Polish Academy of Sciences" (www.if-pan.krakow.pl) is a Polish non-profit Institution well known for brain disease studies and development of novel methods for their treatments. In the laboratories of Prof. K. Wedzony, Head of Pharmacology and Brain Biostructure, several studies are carried out at various experimental stages, from molecular to behavioural level in the field of depression and schizophrenia.

The "Istituto Ricerche Farmacologiche M. Negri" is an Italian non-profit Institution widely recognized in the International scientific community with areas of excellence in the fields of both basic and clinical research. The Institute has developed a range of skills, all coordinated "under the same roof" in order to obtain a comprehensive picture of biomedical problems. Dr. A. Vezzani, Chief of Experimental Neurology Lab., has great experience in biochemical and molecular mechanisms involved in epileptogenesis.

The "Politecnico di Milano" (www.elet.polimi.it) is one of the largest technical universities in Italy, offering Bachelor, Master and Ph.D. courses in Engineering and Architecture. The group coordinated by Prof. G. Gini, covers a broad area of research from micro electronics to telecommunication, from system science to computer science. In particular it is widely recognized for its research in the field of Artificial Intelligence: learning from data, knowledge-based systems, ensembles of classifiers, planning, vision and humanoid robotics.

European benefits and impacts:

The SME partners will gain clear commercial benefits from an industrialization and commercialization of the ION components.
The SMEs prepared a common business plan in order to exploit the expected results worldwide.
Multi Channel Systems gained a much improved commercial system to sell. The new electrophysiological platform developed by this company through the ION project (IONEP) appears definitely more competitive with respect to the other platform present in the market.
SC IT Romania ITR has the intellectual property rights of the new software solutions developed in ION. This software property could give rise to a quite profitable and useful commercial activity in the case of a proper implementation of the ION Business Plan.
Newron and Nikem have the use of the a much improved techniques capable to support in the search for new drugs, which can ultimately be put through clinical trials and eventually to the market.
The RTD partners gained commercial insights and practical experience by working with SMEs on a technical problem of direct commercial significance.

European society, in the long term, will benefit if the project results will increase the speed and efficiency of drug discovery. A major bottleneck in the search for new drugs is the initial screening of the enormous variety of compounds which might, in principle, yield the "blockbuster" medicines of the future.

Other companies and consortia throughout the world are also trying to transform the inherently low throughput of established techniques into high throughput ones.
But the ION project has developed a coherent European solution that will maintain European competitiveness in this important but highly specialised field.

The ION components, after an appropriate industrialization, could find a large market, not only in Europe, but also in external markets such as the US and Japan.

The ION project has an impact on several policies of the EU. Most importantly the project development addresses a number of priorities such as health policy (major diseases), industrial policy (innovative drugs) and economic policy (new technological products).

Project Activities implemented in Months 1-24:

Concerning the consortium management activities, during the twenty-four months of the project execution, the Project Coordinator has implemented and managed the ION Communication and Cooperation system to ensure accurate, timely and effective communication taking place between partners.
A Project Master Document has been approved and adopted by all partners together with some cost control tools.
Five Project Steering Committee Meetings have been hold as planned.

Concerning the RTD and innovation activities, the SME partners have conducted a survey (WP 03), by using a web questionnaire, of end-users of the current screening technologies and identified a clear need for an improved and more efficient screening process.
Overall, the major benefits of the project has been identified in the provision of a single integrated system which can screen candidate compounds more quickly and analyse the results more efficiently than existing alternatives.
The project focused on the development of the specific requirements identified by the end-users within the conceptual framework established in the project's proposal.

The work performed during the project life can be assembled along the following lines:

1. Development of an improved ion channels platform starting from the Roboocyte system, intellectual property of the consortium partner MCS.
   This new platform extends both the engineering profile of the system and the software user interface. The enhanced platform is named IONEP.
   (WP 04)

2. Design and development of the ION Target Library and validation through *in vivo* experiments of the relevance of the selected targets for the pathologies addressed in the project objectives.
   (WP 05)

3. Prototyping of the ION Chemical Library through both the improvement of the database format and the introduction an appropriate annotation system covering a large number of relevant compounds (in relation to the targets addressed in WP 05) recorded in the library (WP 06).

4. Design and development of a prototype of the Sequential Screening Agent performing data analysis and planning of experiments.
   This agent is based on the calculation of the next chemical structure for a given ion channel target. The agent will be applied sequentially starting from structures of ION chemical library.
   (WP 07)

5. Preliminary integration of the ION components (IONEP, target and chemical libraries and software agents) in the ION Sequential Screening System.
   (WP 08)

The role of the RTD partners was the provision of the necessary academic research expertise, and the assistance in selecting the target ion channels and both the candidate and reference drug compounds to be used. Their expertise was also essential in the validation of the outcomes obtain with the new system, ensuring its reliability with respect to the end-users.

Concerning the dissemination activities, during the twenty-four months of the project execution, the ION partners implemented a suite of activities to exploit the achieved results worldwide and prepared the ION Business Plan (WP02).
The quite specific and vertical character of the market for drug screening on ion channels imposed a targeted dissemination activity focused on the informal presentation of the project results at the occasion of scientific events related to the domain.
In addition the delivery of a specialized workshop, held in Milan on 30th November 2006 and open to a wide spectrum of industrial and academic parties was considered necessary to ensure an appropriate communication of the project's achievements to its potential markets and to the scientific community.
A business plan has been prepared carefully by the ION project's SMEs to address the existing potential markets for the ION components and possibly in a later stage for an integrated system.
Accordingly to different market investigations the weak point of all the available ion channels experimental platforms is both the software and the strong proprietary character of the integration hardware-software. ION addresses, with its prototypes, the issue innovatively by providing more comprehensive and powerful software components and by decoupling hardware from software.

Project Results achieved in Months 1-24:

The five most important results achieved during months 1-24 are:

- The **IONEP** (WP 04), that is an improved ion channels platform developed starting from the Roobocyte system, intellectual property of the consortium partner MCS.
  The existing technology based on oocyte recording, uses two separate electrodes to maintain and measure changes in current through ion channels in the membrane. A major innovation of the project is to simplify the existing procedure into a one-electrode system. This is now incorporated into an

improved version of the "Roboocyte" platform produced by MCS in Germany. New software to cope with the data generated by the experimental platform was developed by ITR, a Romanian SME specialized in scientific software.
In addition to analysing the data from initial screening, this software helps in the design of subsequent steps in the drug discovery process.

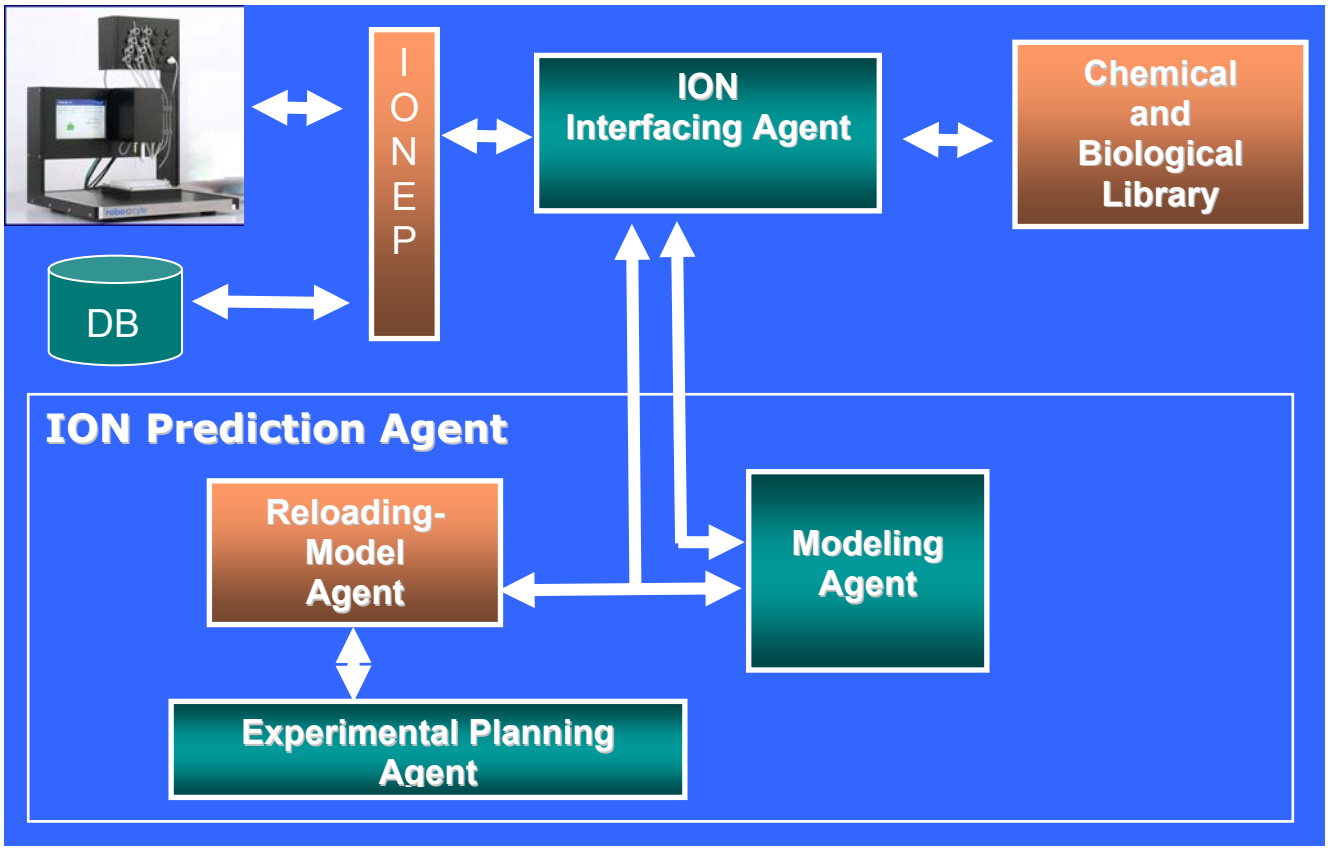
- The **ION Target Library** (WP 05), that defines the suite of most suitable ion channel targets to discover drugs in a given context of diseases (for the ION project Parkinson, depression, neuropathic pain, …). The term annotated library means both a structured and unstructured collection of information and documents describing the nature and properties of the considered cell's membrane proteins (ion channels). The ION Target Library covers a relatively small number of ion channels but it provides a vast annotation including information on the behaviour of these proteins both in vitro and in vivo. The contribution of in vivo data on the considered ion channels is in our view an important outcome of the project.

- The **ION Chemical Library** (WP 06), that is a prototype annotated collection of known chemical structures showing pharmacological activity with respect to the ion channels annotated in the targets library. The chemical structures in the library are organized in a proper ontology. In addition the library provides appropriate codification and formatting software to translate any given structure in a codified data file suitable to be processed by the ION Sequential Screening Software.

- The **ION Sequential Screening Software** (WP 07), that is a software module for the *in silico* estimation of the pharmacological activity (IC50) of a given chemical structure in relation to a specific ion channel target. It works as a suite of QSAR models capable of setting the priority for synthesizing new compounds and planning an appropriate sequence of new experiments. The module implements standard chemical identifiers capable to describe the compounds. The ION Sequential Screening Software is developed on a modeler based on self-organizing ANN, provided by one of the ION project RTD partners.

- The **ION Sequential Screening System (**WP 08), that is a tentative integration of the ION components: IONEP (the experimental platform developed in the first twelve months of the project), Target and Chemical Libraries and Sequential Screening Software.
  It is based on a number of data interchange software modules which can be operated through a common user interface. The system is designed to ensure in a future version the use of different experimental platforms so to be able to address a wider market with respect to the only market of the project's platform (IONEP).

The following diagram represents all the components of the foreseen system and also shows the flow of information during the normal operation of an experimental cycle of prediction and verification of a target value expressed.



Methodologies and approaches employed and achievements of the project to the state-of-the-art:

The current state of the art is a technology that allows the ion channel activity to be measured directly (e.g. two electrode voltage clamp and patch clamp). A number of suppliers of electrophysiology instruments and software deliver systems based on the direct measure of ion channel currents implementing a variety of voltage clamp solutions.
All the systems proposed are focused on the direct measurement of the ion channel currents in a voltage clamp and/or patch clamp setting.
These systems differ in the degree of automation and dimension of the supported experiments, in the quality of the engineering implementation, in the patch clamp electronic details but all share the same type of biological models, the direct channel current measurement.
The state of the art on the software for the management and automation of screening experiment shows the availability of poor (equipments specific) software with no support for data analysis and experiment planning.
The available software is usually bundled to a specific instrument and handles activities such as the injection of the oocytes, the voltage clamp measurement and the wash cycle for the cells.

The main limitation of the present neuronal electrophysiology systems is in their generality which on one hand allows the largest flexibility of use but on the other hand severely limits their applicability to high volume throughput investigations.

The available systems lack two critical components in order to become really effective for drug discovery by means of high throughput screening.
Firstly, they can't offer predefined target libraries and starting molecular structures for selected specific areas of investigation. In the case of the neurological and psychiatric disorders of concern in the ION project these libraries identify a family of membrane proteins (ion channels and GPCR targets) and a collection of molecular structures representing the starting points to support the drug design and the screening process.
Secondly, they do not provide adequate software for a high level of automatic analysis of the experimental outcomes including the suggestion of the best molecular structures for the next step of the screening process. The requirement in drug discovery is a sequential process capable to converge on the best molecular structures to reach its objective with a minimum number of experiments (the shortest sequence).

With respect to the current state of the art, the ION project development introduced two important innovations.

- First, an easy access to a large set of targets and to an efficient drug discovery process, exploiting a well designed and implemented library of starting molecular structures.

- Second, an innovative analysis of the experimental outputs that enable future experiments to be planned more effectively by introducing software agents capable to interpret the outcome of an experiment and to plan the next experiment in a drug discovery screening sequence.

So, the most important innovative feature of ION is introducing a suite of new software tools able to accelerate the analysis of the experimental results and the design of the next screening round for drug discovery in ion channels.
These software agents have been developed for the MCS platform, but they can be adapt to other platforms.

Impact of the project on its industry/research sector:

As above mentioned, the ION potential commercial components are five:

- Drug screening experimental platform (IONEP)
- Molecular Target Library
- Chemical Library
- Sequential Screening Software
- ION Sequential Screening System

Two ION commercial components (the drug screening experimental platform and the ION Sequential Screening System) will be possibly available in the market segment of pharmaceutical industry, biotech companies, universities and research centres.
The competitors in this market are the producers of electrophysiology platforms.

The expected global market volume, including companies and universities/research centres, amounts to 268,75 Millions Euros, with a total need of 1075 instruments worldwide in the next years (2007-2010).
If we estimate that the IONEP and the integrated ION Sequential Screening System can intercept a market share of 8 percent of the global market in the next years, this gives approximately 86 potential customers worldwide, with a total volume of 21,5 million Euros.

One of the most important features of the ION system is that hardware and software components can be commercialized separately. In the technology market where the commercialized products are made of one hardware and one software components (e.g. personal computers), the market dynamic showed a clear advantage (benefit) for those products where the hardware and software were separated (e.g. Microsoft).
So the other ION components (Molecular Target Library, Chemical Library and Sequential Screening Software) will be available separately in a market segment more wide than the previous one. This segment, in fact, includes the pharmaceutical industry, biotech companies, universities and basic research centres, but also the most important electrophysiology platforms producers.
The competitors in this market segment are also potential customers, since they can use the ION user interface software and the ION prediction agent to enhance their platforms.

The expected global market volume for the ION software components, including companies and universities/research centres, amounts to 85,45 Millions Euros, with a total need of 1709 services/software to be provided to users worldwide.
If we estimate that the ION services (Libraries and Software Agents) can intercept a market share of 15 percent of the global market in the next years, this gives approximately 256 potential customers worldwide, with a total volume of 12,82 million Euros.

The integrated systems market for the ion channel screening is currently dominated by US equipments.
European producers are in general SMEs with a strong start-up profile, so having difficulties in developing systems requiring high-level software.
The ION software agents, with their potential of integration with innovative platforms produced by the European start-up, can provide a more general benefit in favour of the European industry in the electrophysiology market.

The fact that software is developed independently from the hardware permits to SMEs producers (mostly European) to market their products as high-level products at a contained price.

Project website:

In order to disseminate the ION project information and achieved results among consortium partners, other interested parties and the general public an appropriate web portal has been implemented.
This portal is currently available at http://www.ion-project.net.

ION Project Contacts:

Mrs Patricia Salvati
ION Project Coordinator
Newron Pharmaceuticals SPA
Via L. Ariosto 21
20091 Bresso (MI)
Italy
Phone: +39 02 610 34601
Fax: +39 02 610 34654
E-mail: patricia.salvati@newron.com
Web Site: http://www.newron.com/

2. DISSEMINATION AND USE

This section describes the publishable results of the "Final plan for using and disseminating the knowledge".

Publishable results

In the following it is provided a short description of each exploitable result that the project has generated.

1. **IONEP: the MCS re-engineered electrophysiology platform with the integration of the high level GUI.**

   Result description / stage of development:

   IONEP is an enhanced experimental platform for ion channel experimental investigation improved on several technical characteristics (single electrode, advanced electrodes, advanced software API).
   It is integrated with an high level experimental preparation, execution and data management graphic software. Its functionality permits a significant increase of experimental productivity in electrophysiology experimental investigation. It also simplify the complexity of experiment planning and execution reducing errors and skill required. It can work with all platform providing an Api over their scripting environment.
   The IONEP platform without the integration of the high level GUI is already marketed by MCS.
   The IONEP high level GUI is in the state of a working prototype. Finalization of the high level GUI into a commercial product is required.

   IPR:
   The integrated new platform remains intellectual property of MCS.
   The software is an intellectual property of ITR.

   Contact details:
   Multi Channel Systems GmbH:
   Mr. Karl-Heinz Boven (boven@multichannelsystems.com)
   IT Romania ITR SRL:
   Mr. Bruno Bottini (bottini@itr.ro)

2. ION Target Library

Result description / stage of development:
The targets library is an annotated database of ion channel targets built starting from a proto-library of NWP complemented by data and knowledge of the ION RTD partners.
The ION target library prototype needs improvement in the managing software and clarification for what concerns the IPRs.
NWP own the major part of this library and it has not yet decided about the finalization and commercialization of a products.

IPR:
The library is an intellectual property of NWP.

Contact details:
Newron Pharmaceuticals SpA:
Mrs. Carla Caccia (carla.caccia@newron.com)

3. ION Chemical Structures Library

Result description / stage of development:
The chemical library is an annotated database of chemical structure presenting specific activities on ion channel targets. The chemical structure in the library (Intellectual Property of NWP) are used as starting structure for sequential experiments.
The ION chemical structures library prototype needs improvement in the managing software.
NKR owns the database DEMO format and it has not yet decided about the finalization and commercialization of a products.

IPR:
The database DEMO format is an intellectual property of NKR.

Contact details:
Nikem Research Srl:
Mr. Massimo Dondio (massimo.dondio@nikemresearch.com)

4. ION Sequential Screening Software

Result description / stage of development:
The ION sequential screening software is a high level software agent for drug discovery through the execution of a suite of sequential experiments. It works with both ION targets and chemicals libraries.
The ION sequential screening software is in the state of a working prototype and a detail plan for its development in a commercial products is required.

IPR:
Intellectual property is acquired by ITR.
The software is now in the first phase of copyright protection. Exploitation possibly through licensing.

Contact details:
Newron Pharmaceuticals SpA:
Mrs. Carla Caccia (carla.caccia@newron.com)
Nikem Research Srl:
Mr. Massimo Dondio (massimo.dondio@nikemresearch.com)
IT Romania ITR SRL:
Mr. Bruno Bottini (bottini@itr.ro)