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MEDITRANS

Targeted Delivery of Nanomedicine

Integrated Project

Sixth Framework Programme, Priority 3; NMP

Periodic activity report

Publishable executive summary

Period covered: from 01/01/2007 to 31/12/2007	Date of preparation: 14/02/2008
Start date of project: 01/01/2007	Duration: 48 Months
Project co-ordinator name:	Prof. Dr. Gert Storm
Project co-ordinator organisation name:	Utrecht University

PUBLISHABLE EXECUTIVE SUMMARY

Project objectives

- To demonstrate the potential of *Emerging Materials* (carbon-based nanoparticles) for use as carrier materials in targeted nanomedicines
- To develop highly effective nanomedicines based on *Candidate Materials* (already used in proof-of-concept drug delivery studies in animals) by virtue of improved targeting and drug release properties
- To promote the entry of nanomedicines based on *Established Materials* into industrial exploitation activities and clinical proof-of-concept studies
- To develop high-sensitivity imaging probes properly designed for guiding drug delivery processes *in vivo*
- To formulate proprietary industrial drug molecules, already established drugs, and DNA- or RNA-based drugs into targeted nanomedicines with well-characterised and optimised physicochemical properties
- To optimise the targeting efficiency of the nanomedicines under development by *in vitro* target recognition studies
- To improve the intracellular targeting of siRNA/pDNA-loaded nanomedicines in cancer and endothelial cells
- To maximise the drug availability at the target site by means of external physical stimuli that induce drug release from the targeted nanoparticles 'on demand'
- To develop targeted nanomedicines from which release of drug/imaging probe is promoted by physiochemical characteristics of the pathological microenvironment
- To develop imaging procedures for the monitoring of the various steps in the targeted drug delivery process (nanoparticle targeting and accumulation, drug release, local level of drug and of biomarkers in response to therapy) by means of 'smart' imaging probes
- To optimise biodistribution, targeting efficiency, and therapeutic activity of the nanomedicines under development in suitable animal models of rheumatoid arthritis, Crohn's disease, multiple sclerosis and cancer
- To assess the toxicological aspects of selected MEDITRANS nanomedicines
- To enter selected prototype nanomedicines into an industrial exploitation phase to evaluate their potential to be developed into a marketable product
- To provide training courses, and access to the GALENOS-Network, provided for consortium scientists, SMEs, and key stakeholders
- To provide effective and efficient dissemination, and demonstration, of the project's results across Europe

Universiteit Utrecht
Netherlands
Prof. Dr Gert Storm
www.pharm.uu.nl/pharmaceutics/
Commissariat À L'Energie Atomique
France
Dr Frédéric Schuster
www.cea.fr
Stichting Biomade Technology
Netherlands
Prof. Dr George Robillard
www.biomade.nl
Magforce Nanotechnologies AG
Germany
Dr Andreas Jordan
www.magforce.com
Charite Universitatsmedizin Berlin
Germany
Prof. Dr Peter Wust
www.charite.de
FOM
Netherlands
Dr Ron Heeren
www.fom.nl
CSEM
Switzerland
Dr Martha Liley
www.csem.ch
PCI Biotech AS
Norway
Dr Anders Hogset
www.pcibiotech.com
Universiteit Gent
Belgium
Prof. Dr Stefaan De Smedt
http://allserv.rug.ac.be
Uniwersytet Łódzki
Poland
Dr Blazej Rychlik
www.uni.lodz.pl
N. V. Organon
Netherlands
Prof. Dr Herman Vromans
www.organon.com
Technische Universiteit Eindhoven
Netherlands
Prof. Dr Klaas Nicolay
www.tue.nl
Molecular Profiles Ltd
United Kingdom
Dr Andrew Parker
www.molprofiles.co.uk
Universität des Saarlandes
Germany
Prof. Dr Claus-Michael Lehr
www.uni-saarland.de
Philipps-Universität Marburg
Germany
Prof. Dr Thomas Kissel
www.uni-marburg.de
Bayer Schering Pharma AG
Germany
Dr Stefan Bracht
www.bayerscheringpharma.de
Across Barriers GmbH
Germany
Dr Eleonore Haltner-Ukomadu
www.acrossbarriers.de
Philips Electronics Nederland B.V.
Netherlands
Dr Holger Gruell
www.philips.com
Merck Serono – RBM
Italy
Dr Beatrice Greco
www.merckserono.net/index.html
Bracco Imaging SpA
Italy
Dr Alessandro Maiocchi
www.bracco.com/Bracco/home.htm
CNRS
France
Dr Chrit Moonen
www.cnrs.fr
Weizmann Institute of Science
Israel
Prof. Dr Michal Neeman
www.weizmann.ac.il
Università Degli Studi di Torino
Italy
Prof. Dr Silvio Aime
www.unito.it
CSIC
Spain
Dr Sebastian Cerdan
www.csic.es
Guerbet S.A.
France
Dr Claire Corot
www.guerbet.com/
University of Copenhagen
Denmark
Prof. Dr Sven Fokjaer
www.farma.ku.dk
Forschungslaboratorien der Philips GmbH
Germany
Dr Jochen Keupp
www.philips.com
Integrated DNA Technologies, BVBA
Belgium
Dr Brian Sproat
www.rna-tec.com or www.idtdna.com
Universidad Nacional de Educacion a Distancia
Spain
Prof. Dr Paloma Ballesteros García
www.uned.es
Infuturia Group AG
Switzerland
Mr Seymour Kurtz / Dr William Dawson
www.infuturiagroup.com / www.infuturia.eu

Work performed, results achieved so far, expected end results and intentions for use and impact:

WP1 WP1 deals with the design and the preliminary evaluation of novel 'Emerging', carbon-based carrier materials (Tasks 1.1 and 1.2), as well as with the optimization of already existing 'Candidate' carrier materials, like polyplexes and polymeric micelles (Task 1.3). Concerning the former part of WP1, several members of UU have visited the CEA in Saclay, and have assisted in detailing and planning the first round of syntheses. Two samples were prepared: as part of Task 1.1.2, a PEGylated fullerene has been prepared (covalent modification; using the Bingel-reaction), and as part of Task 1.1.3, a PEGylated nanotube has been prepared (by means of adsorption and subsequent cross-linking of of PEG2000-based amphiphilic and polymerisable monomers). These compounds have already been forwarded to

BRACCO, who will initiate the *in vitro* cytotoxicity and *in vitro* hemolysis experiments in early 2008. Concerning the second part of WP1, which deals with Candidate Materials and which encompasses 7 in nature quite different and in size quite large subTasks, several of the partners involved in MEDITRANS have already managed to make significant progress, and several manuscripts have already been sent out for publication. Details on the most important advances made with respect to these specific subtasks are described in Section 2 of this report. **Expected end results:** It is expected that at the end of the MEDITRANS project, with some minor modifications, all deliverables and milestones will be completed. This implies that fullerenes and nanotubes will be functionalized and surface-modified, that their toxicity will be assessed. If proven to be suitable, their ability to target diagnostic and therapeutic compounds to disease sites will be evaluated. In addition, this implies that all Candidate Materials evaluated within WP1 (*i.e.* polyplexes, siRNA-containing nanoparticles, molecularly imprinted nanoparticles, polymeric micelles, iron oxide nanoparticles, amino acid-based amphiphilic polymers and stimuli-sesitive liposomes) will be established and properly evaluated, and that the progress made and the insights obtained will further preclinical development. **Intentions for use and impact:** Most of the delivery systems synthesized and characterized in WP1 will be investigated in more detail in other WPs, both *in vitro* and *in vivo*. Those that turn out to hold the most potential for clinical implementation will be assessed in all four diseases addressed within MEDITRANS, *i.e.* in rheumatoid arthritis, in Crohn's disease, in multiple sclerosis and in cancer. It is likely that as compared to standard treatment modalities (*i.e.* untargeted low molecular weight agents), at least some of the nanomedicines developed in WP1 (and further investigated in other WPs) will substantially improve the outcome of the intervention. This will result not only in interesting and high-impact publications, but likely also novel formulation of interest for clinical evaluation.

WP2 The work performed in the reporting period was focused to the achievement of the objectives planned in the first 18 months. The development of highly efficient MRI probes has been started by synthesizing several macrocyclic ligands able to form lanthanide complexes endowed with high thermodynamic stability. The resulting paramagnetic complexes, once loaded in liposomes, can significantly improve the MRI detectability of the vesicle in any MR contrast modes (T_1, T_2 and CEST). Important achievements were obtained in the field of CEST probes, where LIPOCEST agents endowed with highly shifted intraliposomal water protons. This Task was pursued by preparing osmotically shrunken liposomes loaded with paramagnetic shift reagents both encapsulated and incorporated in the bilayer. A further improvement was achieved by encapsulating in the liposome neutral multimers that increase the intravesicular concentration of the paramagnetic centres. The MRI detectability of CEST agents was also facilitated through the development of specific acquisition protocol and post-processing tools. It was also observed that liposomes encapsulating paramagnetic complexes can act as highly sensitive T_2 susceptibility agents. This finding is particularly relevant within this project, because the T_2 effect is related to the integrity of the vesicle and/or to the release of its content. As far as the Emerging Materials is concerned, novel carbon-based nanocarriers loaded with gadolinium and iron were prepared and preliminary characterized *in vitro*. In the field of iron oxide nanoparticles, efforts were primarily devoted to explore new routes for the preparation of long circulating stealth particles. In addition, a protocol for evaluating the sensitivity of iron oxide particle in cell culture was developed. Concerning optical imaging probes, the work has been focused at preparing nanoprobes whose fluorescence could report about the release/stability of the system. To this end, aggregated porphyrins (J-aggregates) were successfully included in nanocarriers based on amphiphilic cyclodextrins and liposomes. **Expected end results:** The results obtained so far are in accordance with the project timeline. **Intentions for use and impact:** The improved MRI agents, as well as the optical imaging probes, developed within the activity of this WP are expected to significantly contribute to the overall impact of the project. MRI and optical probes endowed with improved detectability are to be used in order to facilitate the objectives planned for the other WPs, in particular WP6 to WP9.

WP3 focuses on the formulation and characterisation of nanocarriers loaded with drugs and imaging agents. This first year has predominantly concentrated on the formulation and characterisation of Established nanocarrier systems, including long-circulating liposomes for passive and active drug targeting, PLGA nanoparticles for oral drug targeting and channel protein containing liposomes. To date all these nanocarrier systems have been successfully formulated to contain non-proprietary drugs, such as mesopram, fludarabine phosphate and corticosteroids. Currently, these formulated nanocarriers are being characterised with regards to their size, charge, drug loading and release profiles. **Expected end results:** Those formulations that demonstrate favourable physicochemical properties will proceed forward to *in vitro* testing and ultimately *in vivo* animal studies. **Intentions for use and impact:** The nanocarriers are being loaded with drugs to ultimately treat medical conditions such as cancer, Crohn's disease, MS and RA. Those currently being testing are mesopram to treat Crohn's disease, fludarabine phosphate to treat cancer and corticosteroids to treat cancer, CD and MS. This coming year will see the formulation of proprietary drugs. The incorporation of imaging probes (developed in WP2) will enable MRI-guided drug delivery visualisation.

WP4 Selection of folate receptor expressing ovarian cancer cell line; Preparation and partial characterization of mesopram loaded PLGA nanoparticles; Induction of inflammatory response in the intestinal cell line Caco-2 using lipopolysaccharides from different enterobacteriae; Growth of cells on silicon nitride membrane wafers provided by CSEM; Optimization of PBEC culture in preparation of the inflamed blood brain barrier model. **Expected end results:** *In vitro* screening systems of nanocarrier extravasation ability and active targeting potential; Well characterized, stable *in vitro* screening systems representing the inflamed colonic mucosa and the blood brain barrier

in the state of inflammation; A miniaturized cell support system with integrated resistance measurement. **Intentions for use and impact:** Patent application and commercial distribution of the miniaturized cell support system.

WP5 In order to improve the intracellular delivery of therapeutic nucleic acids by nanoparticles it is important to understand their behaviour in cells. Thus, WP5 will contribute to the project by focussing on the quantification of the intracellular steps involved in the delivery of siRNA and pDNA loaded nanoparticles (WP1, WP3) to endothelial and tumour cells. As nanoparticles are generally taken up by cells by an endocytic pathway, and thus have to be able to escape from endosomes, a major focus will be on technologies, which can enhance the escape of nanoparticles from endosomes. Endosomal escape of siRNA and plasmid DNA loaded nanoparticles by "Photochemical Internalisation" (PCI) will be especially studied. WP5 was active during the last two months of 2007. A number of activities were launched. (a) Research on the intracellular uptake of nanoparticles, especially to reveal by which endocytosis route they are internalized, was initiated. (b) Research on the intracellular behaviour of biodegradable siRNA loaded nanogels was started (siRNA mediated knockdown of EGFR in A431 cells). (c) Microscopy-based methods to study the dissociation and aggregation of MEDITRANS nanoparticles in full serum/blood were further developed. (d) The collaboration between GHENT and MARBURG on MEDITRANS nanocarriers was initiated through a number of tele-meetings. **Expected end results:** It is expected that by the end of MEDITRANS all deliverables and milestones will be completed. This implies that based on the results in the initial phase of WP5, feedback will be given to WP1 and WP3 regarding the properties of the nanocarriers, which should be further improved to optimise their intracellular trafficking. Feedback will also be given to, and received from, WP4 and WP6 regarding therapeutic targets (WP4) and the development of externally triggered drug activation (WP6), especially regarding light-induced activation. **Intentions for use and impact:** Optimised nanoparticles emerging from WP5 will be delivered to WP9 for testing in *in vivo* cancer models, both alone and in combination with the PCI technology for site-specific drug activation.

WP6 WP6 has started its activities in Month 8. The work performed in the reporting period was focused towards the two Tasks of WP6 in this reporting period: 1) To assess the differences between the spontaneous release of the nanocarrier payload and the release activated by physical means such as ultrasound radiation, or by increasing the local temperature. 2) To assess the differences between the spontaneous release of the nanocarrier payload and the release activated by endogenous activators such as pH and specific enzymatic activity. In the reporting period, the results obtained within the WP6 activity have established a solid basis for achieving the expected objectives of the project. With respect to external stimuli, the use of MRI and Ultrasound in the field of local drug delivery has been reviewed (paper in press). Synthesis and *in vitro* characterization of novel temperature-sensitive drug delivery vehicles is reported. Novel iron oxide particles have been synthesized and tested for use in either detection of drug delivery, but also for local heating by induced currents with innovative method of alternating magnetic field. Several novel MR contrast agents (based on paramagnetic ions, or on 19F) have been designed as reporters for drug release from nanoparticles. Progress is reported with respect to novel contrast agents for methods based on Chemical Exchange of the reporter with water (CEST, PARACEST). The first *in vivo* applications of ultrasound destruction of ultrasound contrast agents in the presence of macromolecular MR contrast agent have been performed on rat liver monitored by MRI. With respect to internal stimuli, much attention has gone in the synthesis and *in vitro* characterization of pH sensitive liposomes, and in the monitoring of drug release *via* MR contrast agents (similar to those reported above), as well as optical contrast agents. Synthesis and *in vitro* characterization is reported for a novel remote controlled valve as a new drug release mechanism, based on a pore-forming bacterial membrane protein. Drug delivery monitoring tests have been performed on cell suspensions based on optical and MRI methods. **Expected end results:** The results obtained are in accordance with the project timeline and Deliverables and Milestones will be completed. **Intentions for use and impact:** The improved drug delivery vehicles, drug delivery methods, contrast agents as reporters of drug delivery, and the start of their applications *in vitro*, in cell cultures, and *in vivo* are expected to significantly contribute to the overall impact of the project.

WP7 Oral targeting of PLGA-based nanomedicines to treat Crohn's disease was performed. Data from these mesopram loaded nanoparticles look promising and further investigations (*e.g.* to combine therapy with imaging) in an animal model can be done. For that the PLGA NPs need to be equipped with an MRI label. Peptidomimetic cell penetrating peptides (CPPs) of different lengths, chiralities and side groups for cellular uptake and membrane destabilizing properties have been evaluated. Upon positive *in vitro* results with siRNA nanocarriers (silencing TNFα in cell culture models), *in vivo* studies will be performed with selected, optimized formulations. The liposome-based agents that can simultaneously act as highly-sensitive T_2-susceptibility and CEST MRI contrast agents were characterized. Such vesicles can be readily taken up by macrophages and, therefore, may be of interest for the MRI visualization of inflammation sites in rheumatoid arthritis and Crohn's disease. P904 and Sinerem, ferrous oxide nanoparticles, are available for MRI and will be used for image-guided diagnosis, encapsulation in nanocarriers, and validation of effective anti-inflammatory treatment. Collagen-induced arthritis (CIA) is an extensively studied animal model of RA because it shares both immunological and pathological features of human RA and it involves immunization with a known cartilage component. CIA is primarily an autoimmune disease of joints, requiring both T and B cell immunity to autologous type II collagen (CII) for disease manifestation. For the CIA induced mouse model the susceptible mouse strain used is DBA/1. A new 'Plug and Play' radio-frequency coil set-up for *in vivo* MRI studies on RA and CD was successfully developed. MRI tools will be developed for monitoring the progress of

inflammation in RA and for visualization of drug delivery, as well as the therapeutic effects of liposome encapsulated corticosteroids. **Expected end results:** The Deliverables and Milestones are expected to be delivered on time. **Intentions for use and impact:** The Tasks being performed are expected to lead to outcomes contributing significantly to the uses and impact outlined in the Annex I.

WP8 A "smart"imaging probe or also named, pro-contrast agent, sensitive to matrix metalloproteinases (MMPS) action has been produced and tested on biochemical and *ex-vivo* samples from animal models of Multiple Sclerosis demonstrating its sensitivity to increased concentration of MMPs. In parallel the characterization of Experimental Autoimmune Encephalomyelitis (EAE) rat model through conventional MRI and molecular imaging (gadolinium and USPIO) has been carried out. **Expected end results:** The MMP-sensitive contrast agent should be formulated for will direct *in vivo* applications. Targeted nanomedicines will be tested in animal models of MS-like traits. **Intentions for use and impact:** The production of the MMP-smart contrast agent is the first step leading to the formation of an agent combining molecular imaging and drug delivery in inflamed brain sites. The imaging characterization of the EAE model will allow understanding of the localization and time frame of central nervous system disruption as well as to set the ground for *in vivo* imaging monitoring of targeted drug delivery systems.

WP9 The aim of this WP is to study the pharmacokinetics, tissue distribution, targeting efficiency, and therapeutic efficacy of the developed targeted nanomedicines in suitable animal models of cancer. Since the project started, a number of therapeutics were tested showing enhanced efficacy relative to non targeted agents. In addition, significant advances were made towards the use of novel therapies in tumour models. The second focus of this WP is the therapeutic evaluation of MRI-guided drug delivery (triggered release) in animal models of cancer, and the use of MRI and other imaging modalities for analysis of the pharmacokinetics and pharmacodynamics (PKPD) of therapy. Mathematical models were developed to analyze such PK PD data. Novel MRI contrast media were developed and used for initial testing of delivery of nanoparticles in cancer models and for non invasive mapping of the tumour microenvironment. **Expected end results:** The results obtained are in accordance with the project timeline. **Intentions for use and impact:** The results achieved with respect to delivery of particles to tumours and non invasive imaging of particle delivery and the tumour microenvironment, form the foundation for the testing of targeted nanomedicines developed in this project.

WP12 Task 12.1 D3 Initial advanced drug delivery course held and evaluated is now due in Month 14 and is on-track for delivery. This course has been advertised to MEDITRANS partners and is being actively promoted. Sixteen MEDITRANS personnel have registered to attend the course. Task 12.2 Training pages of the public and members' areas of the MEDITRANS website were prepared along with the MEDITRANS forum for scientific exchange. Available training material has been uploaded. Task 12.3 The following has been done (1) development of the Training sections of the website, (2) regular internal advertisement of the Staff Secondment Programme, (3) the collection of the names and contact details of likely secondees (14 names of MEDITRANS PhD students so far), and (4) the collection and collation of completed Staff Secondment Forms. D4 First exchange report was delivered in Month 12. Task 12.4 All MEDITRANS partners have been invited to join the Galenos Network and all MEDITRANS' PhD students have been invited to the next Galenos Event. Other workshop and conference activities have been promoted *via* the MEDITRANS website. D5 Participation in the GALENOS-Network -first report on activities was delivered in Month 12. **Expected end results:** Relevant, up-to-date training provided to MEDITRANS partners implemented *via* courses, website-based information, secondment to other partners labs, and *via* the GALENOS Network. **Intentions for use and impact:** Enable MEDITRANS employees, who participate in WP12 activities, to work as effectively as possible and pass on new skills learned to others.

WP13 Task 13.2 Website maintenance, development and updating throughout the reporting period. In the first reporting period the website has had >6,075 visitors, with >72,232 hits and with >20,633 page views. There have been >27.4 visitors per day, with the USA and The Netherlands being the most active countries. Of the MEDITRANS partners' websites, MARBURG provided most refferals. The confidential contacts database is in preparation and is on schedule. Task 13.3 D2 and D6 First and Second promotional leaflets produced were delivered in Months 4 and 12 respectively. The leaflets are available to download from the website. Other promotional activities included a competition to design the project logo. The best of the 12 designs was by Dr Katrin Fischer from BSP. A project promotional poster, produced in Month 4, introduced MEDITRANS to the research community. It is available to download from the MEDITRANS website. Other project promotional material has also been designed and is available on the MEDITRANS website. **Expected end results:** Dissemination of the non-confidential results from MEDITRANS to scientists, stakeholders, and to the public. **Intentions for use and impact:** Non-confidential results from MEDITRANS will be disseminated.

Main elements of the publishable results of the Plan for Using and Disseminating Knowledge:
No publishable results of the PUDK are available at this stage of MEDITRANS.

Prof. Dr. Gert Storm (Co-ordinator)
Universiteit Utrecht
Utrecht, The Netherlands
Tel: +31 30 2537306
Fax: +31 30 2517839
Web: www.pharm.uu.nl/pharmaceutics