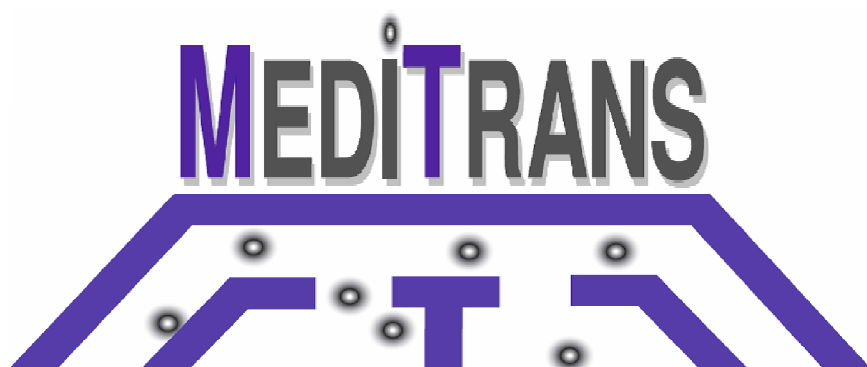
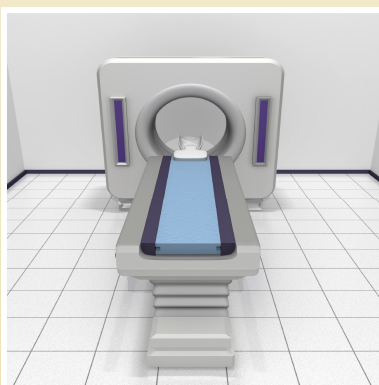
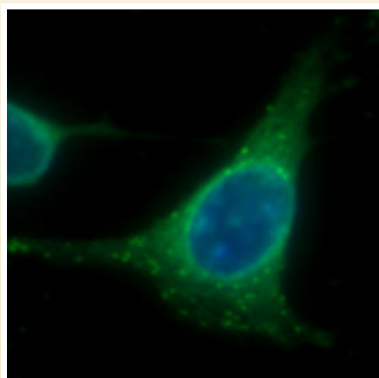
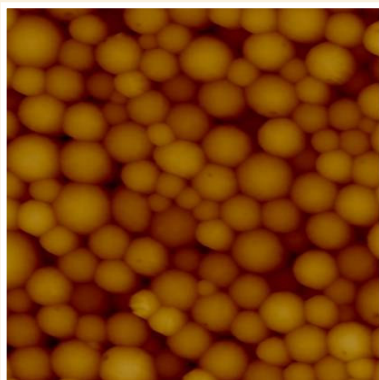
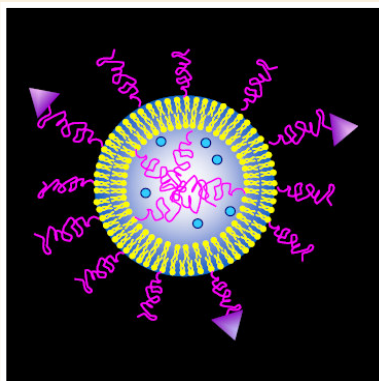




# Targeted Delivery of Nanomedicine

[www.meditrans-ip.net](http://www.meditrans-ip.net)



An Integrated Project funded by the European Commission under the 'nanotechnologies and nanosciences, knowledge-based multifunctional materials and new production processes and devices' (NMP) thematic priority of the Sixth Framework Programme

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**Cover images courtesy of:** **1:** Dr R. Schiffelers (Utrecht University), **2:** Prof. Dr C.-M. Lehr (Saarland University) **3:** Dr K. Fischer (Bayer Schering Pharma AG), **4:** www.istockphoto.com, **5:** From: Dousset V, Brochet, B, Deloire MSA, Lagoarde L, Barroso B, Caille J-M, Petry KG. MR imaging of relapsing multiple sclerosis patients using ultra-small particle iron oxide and compared to gadolinium. AJNR - Am J NeuroRadiology 2006; 27(5):1000-5.

## Work Package 1: Nanocarrier Design

### Objectives

- To demonstrate the feasibility of the Emerging Materials (carbon-based nanoparticles) for targeted drug delivery
- To optimise the functionality of the Candidate Materials (polyplexes, nanogels, nanospheres, polymeric micelles, molecular imprinted particles, stimuli-sensitive liposomes) for targeted drug delivery

### Work plan and conclusions from the first year

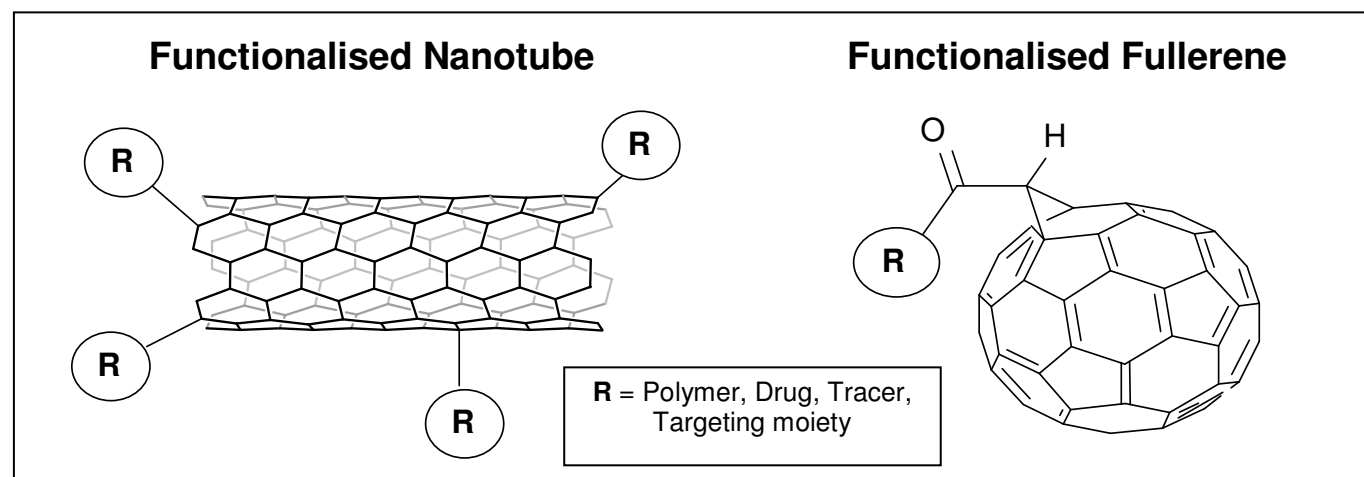
Work Package 1, Nanocarrier Design, aims to identify materials which are suitable for serving as drug delivery devices. On the one hand it deals with the design, synthesis and the physicochemical / toxicological evaluation of novel carrier systems, like fullerenes and carbon nanotubes (*i.e.* the so called emerging materials), as well as, on the other hand, with an optimisation of already partly established systems, *i.e.* the so called candidate materials, like cationic polyplexes and polymeric micelles.

Work Package 1 is subdivided into three different lines of research. In Task 1.1, Utrecht University and members from the CEA in Saclay have set out to synthesise several batches of functionalised carbon nanotubes, which will be surface-modified with different types of polymers ( $\Rightarrow$  to assist the constructs in achieving proper circulation times and target site concentrations). The first rounds of syntheses have recently been completed, and the first sets of nanotubes are currently being prepared for further characterisation. In the upcoming 6-12 months, CEA will further fine-tune the synthesis of the

nanotubes, introducing *e.g.* various chemical groups to more easily functionalise the carriers at later stages of the project. UU will, in the meantime, evaluate the physicochemical properties of the carbon-based carrier-materials, and later on potentially also their pharmacokinetics and their biodistribution.

To follow-up on this, as part of Task 1.2, BRACCO and CEA will, in the next year, initiate attempts to address the safety aspects of functionalised nanotubes and/or fullerenes. This will be done by determining their *in vitro* cytotoxicity against a variety of different cell types, as well as by investigating their *in vitro* haemolysis and their plasma component association. Furthermore, and likely most importantly, the (acute) *in vivo* toxicity of the carbon-based carrier systems will be assessed, in order to identify any potentially serious safety concerns as early as possible.

The third, quite different, Task of Work Package 1 focuses exclusively on candidate materials. Ten different consortium members participate in this Task, and they together aim to intensively investigate and improve already partly established delivery systems, like DNA- and siRNA-containing polyplexes, molecularly imprinted nanoparticles, long-circulating polymeric micelles, and stimuli-responsive liposomes, designed to release their contents upon exposure to external physical triggers, like light, ultrasound or hyperthermia. In the first two years of MEDI TRANS, the WP1 partners will primarily focus on the synthesis, the physicochemical characterisation and the *in vitro* evaluation of the carrier-drug constructs, and in the subsequent two years, the pharmacokinetics, the biodistribution and the therapeutic aspects of the nanomedicines will be emphasised.



**WP Leader:** UU - Prof. Dr Gert Storm (g.storm@uu.nl)

**WP Participants:** UU, CEA, BIOMADE, MAGFORCE, GHENT, MARBURG, RBM, BRACCO, UNITO, CU

## Work Package 2: Development of High Sensitivity Imaging Probes for Guiding Drug Delivery Processes

### Objectives

- To develop high relaxivity Gd-agents including probes responsive to tissue microenvironmental pH and specific enzymatic activities
- To develop high sensitivity Chemical Exchange Saturation Transfer (CEST) agents including probes able to report about the level of drugs or specific biomarkers within the pathological region
- To develop novel iron oxide particles properly designed for applications in drug delivery processes
- To develop highly sensitive Optical Imaging probes for monitoring drug delivery processes and therapeutic effects

### Work plan and conclusions from the first year

Highly sensitive paramagnetic Gd-loaded liposomes encapsulating anti-tumoural drugs (*e.g.* Doxorubicin) and properly functionalised for targeting specific receptors overexpressed in tumours (*e.g.* NCAM receptors) have been prepared and tested both *in vitro* and *in vivo* in a tumour mouse model for assessing their potential for guiding drug delivery processes (see Fig. 1 below on the left).

Novel responsive agents towards pH, temperature and enzymatic activity (matrixmetalloproteases) have been synthesised and tested *in vitro*.

Highly-sensitive paramagnetic liposome-based CEST agents (LIPOCEST) endowed with highly shifted intraliposomal water protons were prepared. This Task was accomplished by preparing liposomes whose aqueous core is hypotonic. The osmotic stress occurring when the liposomes are dispersed in an isotonic medium shrinks the vesicles, inducing a change in their original spherical shape (see Fig. 2). LIPOCESTs are loaded with paramagnetic lanthanide(III) shift reagents that, when entrapped in a non-spherical compartment, increase the paramagnetic shift of the intraliposomal water protons through the so-called Bulk Magnetic Susceptibility effect.

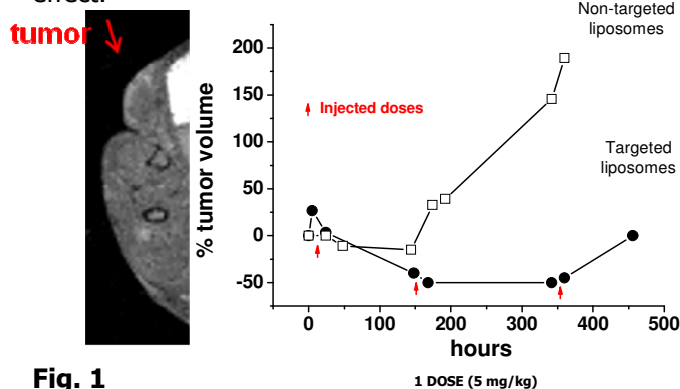


Fig. 1

This contribution is also affected by the orientation of the non-spherical vesicles in the magnetic field, and this property can be nicely modulated by changing the magnetic properties of the amphiphilic metal complex incorporated in the liposome bilayer. In such a way, LIPOCEST agents with resonance frequency of their intraliposomal water protons ranging from -50 to +20 ppm have been prepared. A further increase in the chemical shift values have been pursued by encapsulating polynuclear neutral shift reagents that increase the overall concentration of paramagnetic complexes inside the vesicles without increasing the osmolarity in the inner cavity. The extension in the resonance frequency values of these nanoprobe may substantially facilitate their *in vivo* multiplex MR visualisation.

Preliminary experiments demonstrating the potential of CEST MR contrast for guiding drug delivery have been carried out by preparing liposomes encapsulating a PARACEST agent. By selecting a liposome bilayer with low water permeability the encapsulated probe appears silent in a CEST MR experiment, but it can be visualised by applying an external stimulus (*e.g.* low frequency US) which is able to induce the release of the metal complex.

Using the laser pyrolysis method, novel iron-based nanoparticles have been prepared.

TEM measurements showed ultra small particles (iron, iron oxide, iron carbide, *etc.*) embedded in carbon. A complete physico-chemical characterisation of the nanopowders is ongoing.

The development of highly-sensitive probes for Optical Imaging has been pursued by preparing liposomes containing quenched fluorescent dyes (*e.g.* aggregated porphyrins) that can switch to fluorescence upon exposing the vesicle to a specific external (temperature, US, light) or internal (pH, enzyme, *etc.*) triggering stimulus able to promote the release of the material entrapped in the vesicle.

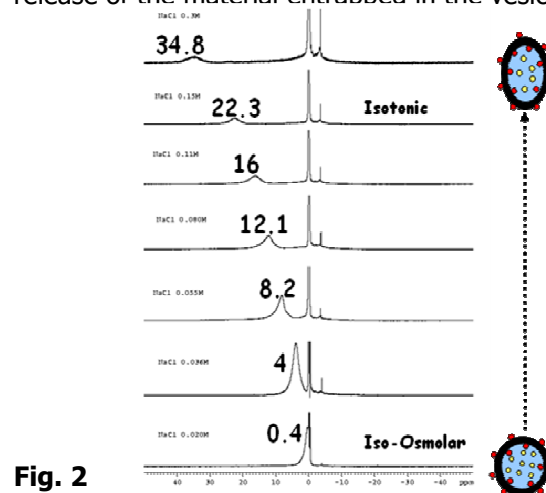


Fig. 2

**WP Leader:** UNITO - Prof. Dr Silvio Aime (silvio.aime@unito.it)

**WP Participants:** CEA, MAGFORCE, TUE, PHILIPS, BRACCO, UNITO, CSIC, GUERBET, PHILIPSD

## Work Package 3: Formulation of Drugs and Imaging Agents into Carriers / Physicochemical Characterisation

### Objectives

- To load the nanocarriers with biologically active compounds and imaging agents
- To study the physicochemical characteristics of the nanomedicines which are relevant for the overall aims of MEDITRANS

### Work plan and conclusions from the first year

Work Package 3 aims to load a range of different nanocarriers with drugs and imaging agents, and to optimise their physicochemical characteristics. The selected drugs are to include anti-inflammatory drugs to treat Crohn's disease, low molecular weight anti-MMP drugs to treat MS, corticosteroids to treat cancer and chronic inflammatory conditions, low molecular weight drugs to treat cancer, siRNA and pDNA. Imaging agents developed in WP2 will be used for MRI-guided drug delivery visualisation.

Work Package 3 is sub-divided based on the nanocarrier system. Task 3.3 (Months 4-48) focuses on the optimisation of already established systems, for example, long-circulating liposomes and PLGA nanoparticles. Task 3.2 (Months 12-48) focuses on determining the viability and optimisation of candidate nanocarriers, ranging from nanoparticles for the delivery of siRNA to stealth-coated iron-oxide particles. Task 3.1 (Months 12-24) details the coupling of drugs and imaging agents to emerging nanocarrier systems developed in WP1, such as nanotubes and fullerenes.

The last 8 months has seen the recruitment of PhD students to initiate and proceed with the formulation and physicochemical characterisation of established nanocarrier systems. A drug selection meeting was held in Utrecht, in which non-proprietary drugs were chosen with respect to the formulation of both established (Task 3.3) and candidate (Task 3.2) nanocarrier systems.

For the established systems, promising results have been obtained using long-circulating liposomes loaded with different types of corticosteroids. Early indications suggest that one of the primary mechanisms for anti-tumour efficacy is the inhibition of the production of pro-angiogenic factors by tumour-associated macrophages. Work is on-going. The active targeting of long-circulating liposomes to

MS lesions, is currently being initiated aided by the recruitment of a new PhD student. The development of liposomal channel proteins loaded with corticosteroids has been planned and will be realised through the collaboration of BIOMADE with the UU. The formulation and characterisation of PLGA nanoparticles loaded with mesopram (anti-inflammatory to treat Crohn's disease) for oral drug delivery has been initiated and is on-going.

The next 12 months will see the formulation and characterisation of the established systems containing proprietary drugs provided by industrial partners, but also candidate systems. In addition, during this period the physicochemical properties of carbon-based materials (evaluated in WP1) will be studied.

**WP Leader: MOLPROF** - Dr Andrew Parker (aparker@molprofiles.co.uk)

**WP Participants:** UU, BIOMADE, MAGFORCE, FOM, GHENT, ORGANON, MOLPROF, UDS, MARBURG, BSP, PHILIPS, RBM, UNITO, CU, PHILIPSD, IDT

## Work Package 4: Recognition of Targets: Cells, Tissues, Organs

### Objectives

- To optimise the targeting efficiency of the nanomedicines with respect to their disease specific application (cancer, rheumatoid arthritis (RA), Crohn's disease (CD), multiple sclerosis (MS))
- To explore and optimise new principles of active, ligand-mediated targeting (*e.g.* to angiogenic endothelial cells in tumours and RA, to myelin and ICAM-1 in MS lesions)
- To transfer the principle of passive targeting ("EPR effect") to new disease specific applications (*e.g.* targeting to inflamed colonic mucosa in Crohn's disease, and to MS lesions in MS)
- Development of disease-specific *in vitro* models of cellular targets and barriers, reflecting relevant pathological changes (*e.g.* intestinal mucosa and blood brain barrier, respectively, in the state of inflammation)

### Work plan

Work Package 4 aims to aid in the optimisation of nanoparticulate drug formulations (supplied by WP3) by screening for active / passive targeting potential and extravasation ability and to provide a better understanding of disease specific changes in relevant biological barriers.

In binding assays the drug and siRNA nanocarriers will be evaluated for their targeting ability to disease specific ligands such as ICAM-1 and MMP in the case of multiple sclerosis. To determine extravasation ability a novel assay will be established, correlating the *in vitro* binding of serum proteins to nanocarriers with their *in vivo* circulation half time. The determination of serum protein binding will allow for a rapid screening of stealth particulates.

To test the targeting specificity of nanomedicines directed against Crohn's disease and multiple sclerosis and better understand the mechanism of their uptake into, or transport across the inflamed tissue, *in vitro* models for the two biological barriers involved in the state of inflammation are to be developed.

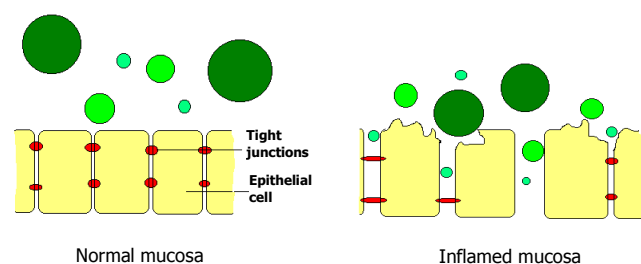


**Fig. 1: "Intestinal EPR (Enhanced Permeability and Retention) effect"**

In the intestinal model an inflammatory response could be induced in the colon carcinoma cell line Caco-2 using lipopolysaccharides from both *Escherichia Coli* and *Salmonella typhimurium*. The effect, monitored *via* IL-8 and TNF-Alpha expression levels, was time and concentration dependent.

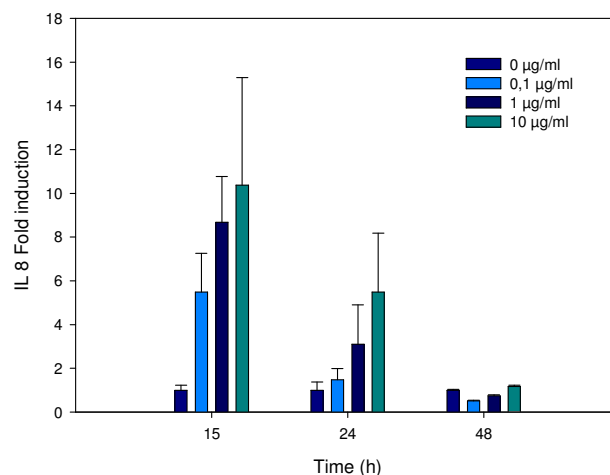


**Fig. 2: IL8 level after stimulation of Caco-2 C2Bbe1 with LPS from *S. typhimurium***

To allow higher throughput of experiments and to incorporate automated resistance measurements in the system, Caco-2 cells were grown on silica wafers supplied by CSEM. The wafers as well as the silicon seals used to divide the system into several compartments provided a good support for epithelial cell growth, showing no apparent cytotoxic effects. The setup will be characterised in detail with regards to cell morphology, barrier properties and transport behaviour of wafer grown cells.

In an approach similar to the inflamed colonic mucosa model, inflammatory response will be induced in porcine brain endothelia cells to simulate the blood brain barrier in the state of inflammation. The model will be characterised with regards to morphology, barrier properties, expression of drug relevant transport proteins and transport of CNS relevant drugs across the inflamed model.

### Conclusions from the first year

Epithelial and endothelial cell lines grown as monolayers on permeable supports show time and dose dependent inflammatory responses upon external stimuli. This approach holds promise for the development of a reproducible *in vitro* test system to study the mechanisms of site-specific targeting of nanoparticulate drug carriers to sites of inflammation.

**WP Leader:** UDS - Prof. Dr Claus-Michael Lehr (lehr@mx.uni-saarland.de)

**WP Participants:** UU, MAGFORCE, CHARITE, CSEM, UDS, BSP, ACROSS, RBM, WEIZMANN

## Work Package 5: Target Cell Uptake and Intracellular Trafficking

### Objectives

- Contribute to the understanding of the (biophysical) behaviour of siRNA / pDNA nanoparticles in cancer and endothelial cells
- Especially, identifying the most critical step in the delivery of siRNA / plasmid DNA to the target cells, which may strongly depend on the architecture of the nanoparticles
- Use this knowledge to allow WP1 to design nanoparticles which successfully deliver *in vivo* nucleic acids to cancer and endothelial cells
- Design and synthesise nanoparticles that can be used for light-triggered intracellular delivery of nucleic acids

### Work plan

Cationic biodegradable microgels based on dextran were prepared through an emulsion photopolymerisation method. The advantage of such cationic hydrogel particles is that they can be loaded with siRNA posterior to gel formation. Thus, the exposure of siRNA to free radicals, UV light and shear

stress during particle formation is avoided. Even though the incorporation of siRNAs in the microgel network is based upon electrostatic interaction, still a controlled release can be achieved in function of hydrogel degradation.

To demonstrate the biological functionality of these siRNA loaded microgels, preliminary cell culture experiments on cationic dextran microgels (mean diameter  $\sim 2\text{-}3\text{ }\mu\text{m}$ ) in a human cancer (HUH7) cell line were performed. Confocal microscopy revealed that the microgel particles were easily internalised by the cancer cells. Moreover, cationic microgels loaded with siRNA targeted against enhanced green fluorescent protein (EGFP) showed significant silencing of the EGFP gene, when compared to microgels containing negative control siRNA.

### Conclusions from the first year

We conclude that the dextran microgels are internalised by cells and release active siRNA inside the cytosol. Altogether, these data are encouraging for the application of siRNA loaded cationic hydrogels for the intracellular release of siRNA.

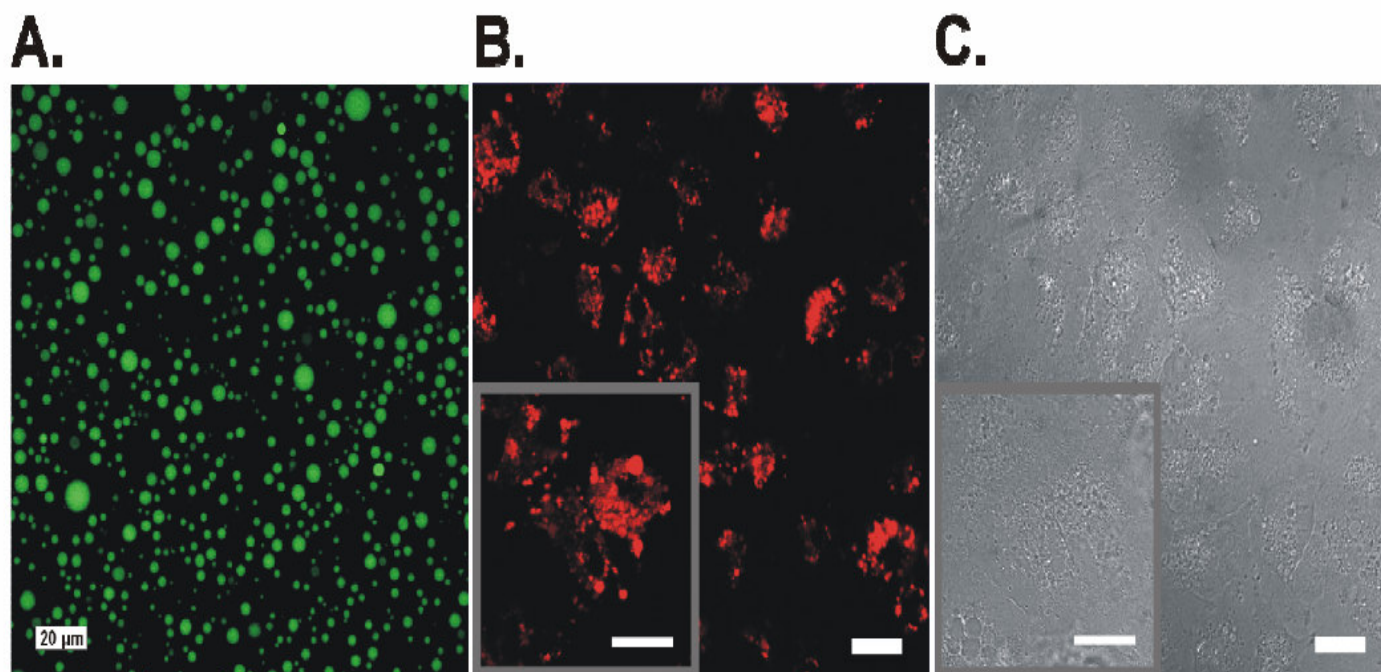


**Fig. 1: (A) Confocal micrograph of cationic dextran microgels loaded with green (atto488) labelled siRNA, (B) confocal fluorescence image and (C) corresponding transmission image showing the internalisation of the microgels, loaded with red (atto647) labelled siRNA, in cancer cells**

**WP leader:** GHENT - Prof. Dr Stefaan de Smedt (stefaan.desmedt@ugent.be)

**WP Participants:** UU, PCI, GHENT, MARBURG, UNITO

## Work Package 6: Stimulus Induced Release / Activation

### Objectives

- To maximise availability of nanoparticle-bound drugs to target cells by using external stimuli to induce drug release from the targeted nanocarriers 'on demand'
- To optimise the release of the drug / imaging probe payload from the nanocarrier in response to physicochemical characteristics of the biological microenvironment
- To develop MRI procedures for the quantitation of *in situ* drug availability and delivery by means of "smart" imaging probes

### Work plan

WP6 aims to explore ways to trigger the release of drugs from nanocarriers localised within the pathological region by means of external stimuli ("release at will") or by exploiting specific physicochemical characteristics of the pathophysiological microenvironment (internal stimuli). The WP is divided in to two different lines of research: externally applied stimuli, and endogenous stimuli. The third objective (development of MRI procedures) is common to both lines of research.

The first line of research contains two elements: release activated by mechanical action (based on ultrasound), or by increasing the local temperature (ultrasound alone or oscillating magnetic fields in the presence of iron oxide particles at the targeting site). The use of ultrasound (US) for triggered drug release from nanocarriers, as well as the role of MR contrast agents in US triggered drug delivery has been reviewed by CNRS at the start of WP6 (Deckers *et al.* JMRI, in press) emphasising not only local drug release from nanocarriers, but also the multiple US-tissue interactions relevant for drug delivery (cavitation, increased drug extravasation, membrane permeability). Preliminary studies on the use of US contrast agents for increased cavitation has demonstrated enhanced uptake of macroscopic MR contrast agent in rabbit liver (collaboration CNRS-GUERBET). MAGFORCE is developing optimised protocols for local heating by oscillating magnetic fields in the presence of iron oxide particles.

The second line of research is centered on the drug release from nanocarriers activated by endogenous activators such as pH and specific enzymatic activity. Nanocarriers will be designed, in Sub-Project 1, in

order to show changes in the membrane permeability in response to the microenvironmental characteristics. CSIC has developed a family of pH probes for measuring extracellular pH in tumours non-invasively. The pH measurement protocol can be combined with any drug and nanocarrier identified in SP1 (interaction with WP1) and a suitable tumour model (interaction with WP9).

UNITO has developed several specific MR contrast agents for local drug delivery. These contrast agents, the so-called Chemical Exchange Saturation Transfer (CEST) agents, have the unique property that they can be switched off and on with a second radio-frequency irradiation. These contrast agents may be used with either the first or second line of research.

### Conclusions from the first year

WP6 started in Month 8, and has concentrated on methodological studies awaiting input on nanocarriers from SP1. Ongoing studies have shown significant progress towards the objectives, and have identified external and endogenous release triggering methods and candidate MR contrast agents for local drug delivery.

**WP leader:** CNRS - Dr Chrit Moonen (chrit@imf.u-bordeaux2.fr)

**WP Participants:** UU, CEA, BIOMADE, MAGFORCE, PHILIPS, BRACCO, CNRS, WEIZMANN, UNITO, CSIC, GUERBET, PHILIPSD



## Work Package 7: Application to Rheumatoid Arthritis and Crohn's Disease

### Objectives

- To study pharmacokinetics, tissue distribution, targeting efficiency, and therapeutic efficacy of the developed targeted nanomedicines in suitable animal models of rheumatoid arthritis and Crohn's disease
- To evaluate and optimise targeted nanoparticles, loaded with imaging probes and drugs, for application in MRI guided imaging and therapy of inflammation

### Work plan

WP7 is mainly focussed on three topics, *i.e.* passive and active targeting of nanomedicine and MRI-guided drug delivery. Recruitment and training of staff is ongoing at all centres.

Oral targeting of PLGA-based nanomedicines to treat Crohn's disease has been performed. Histology sections of the TNBS-induced colitis model in the rat are shown below. Data from mesopram loaded PLGA nanoparticles look promising and further investigations (*e.g.* imaging) in an animal model can be done at TUE. For that the PLGA nanoparticles need to be equipped with an MRI label.

Chitosan / Chitosan-modified PLGA Dendrimers (novel + commercial) show promising results. Peptidomimetic cell penetrating peptides (CPPs) of different lengths, chiralities and side groups for cellular uptake and membrane destabilising properties have been evaluated. Upon positive *in vitro* results with siRNA

nanocarriers (silencing TNF $\alpha$  in cell culture models), *in vivo* studies will be performed with selected, optimised formulations.

P904 and Sinerem ferrous oxide nanoparticles are available for macrophage targeting in cell or animal models, encapsulation in nanocarriers, validation of effective anti-inflammatory treatment, *etc.* A new 'Plug and Play' MRI RF coil set-up for rheumatoid arthritis and Crohn's disease was successfully developed at TUE.

Future studies are planned with new, emerging and approved drugs, along with the introduction of targeted particles. Drugs that will be used within Work Package 7 are corticosteroids (Uu), siRNA (IDT), Mesopram (BSP) and anti-MMP (RBM). Animal model studies for Crohn's disease will be performed at Uds and at TUE and for rheumatoid arthritis, Uu and TUE will build up a facility in collaboration with Nijmegen University. PCI will be used as a technique that may aid in the cytosolic delivery of liposomal materials and local (intra-articular) delivery and systemic delivery of PEGylated carriers. Pharmacokinetic / biodistribution studies will be performed with selected, optimised formulations.

### Conclusions from the first year

Encouraging preclinical results were obtained for Crohn's disease with corticosteroids. MRI tools will be developed for visualisation of drug delivery and for monitoring the effect of treatment.

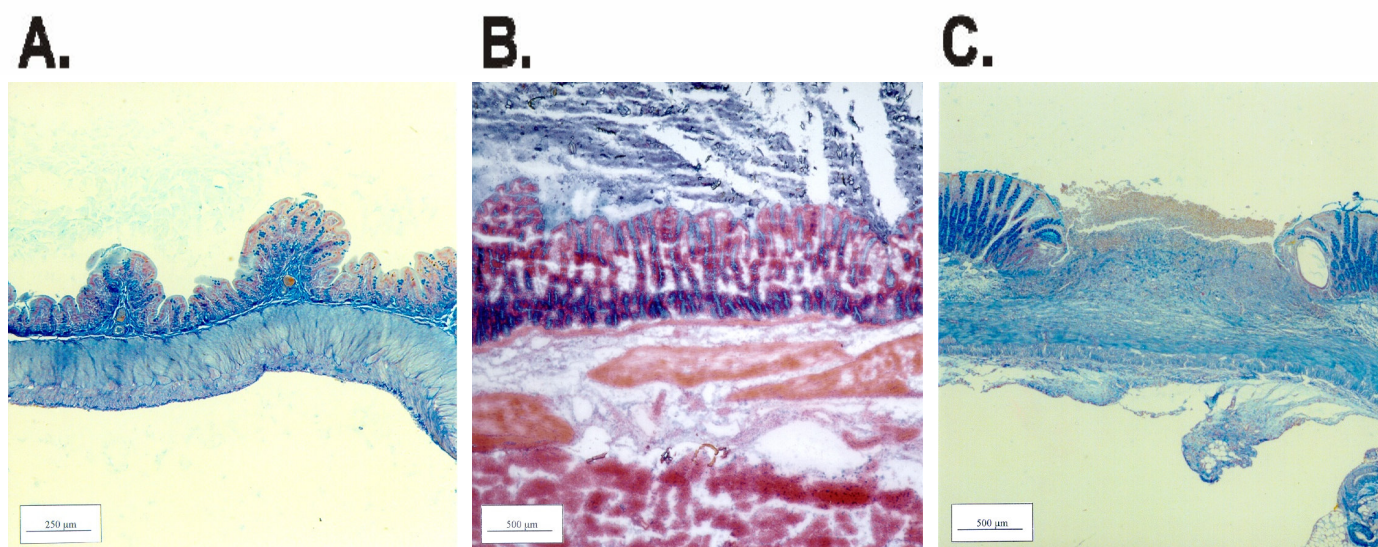


**Fig. 1: (A) Healthy control group, (B) colitis group with increased mucus production, (C) colitis group with ulcerated tissue**

**WP leader:** TUE - Prof. Dr Klaas Nicolay (k.nicolay@tue.nl)

**WP Participants:** UU, FOM, TUE, UDS, BSP, PHILIPS, RBM, UNITO, CSIC, GUERBET, CU, PHILIPSD, IDT

## Work Package 8: Application to Multiple Sclerosis

### Objectives

- To study pharmacokinetics, tissue distribution, targeting efficiency, and therapeutic efficacy of the developed targeted nanomedicines in suitable animal models of multiple sclerosis (MS)
- To design and optimise a carrier for imaging-guided drug release and delivery in the central nervous system (CNS)
- To evaluate, *in vivo*, the therapeutic efficacy of matrixmetalloproteinases (MMP)-inhibitors, through imaging-guided targeted and triggered delivery, in CNS lesions induced by MS-like pathology

### Work plan

A Gadolinium-based pro-contrast agent (proCA) sensitive to a family of MMP active enzymes has been synthesised and tested *in vitro*: DOTA-gadolinium has been covalently linked to a peptidic sequence that is a substrate for some MMPs, and to an insolubilising moiety. This assembly constitutes a prodrug form of the CA (*i.e.* the proCA). The peptide part in the proCA is released from the carrier upon cleavage by MMPs at the site of action, thus activating an MRI signal in T1-weighted images. The insolubilising moiety in the proCA, which suppresses any T1-enhancement, makes these particles T2-relaxation agents in T2-weighted image acquisitions. This proCA has been tested in biochemical assays as well as in *ex-vivo* blood samples from a disease animal model mimicking aspects of multiple sclerosis, the experimental autoimmune encephalomyelitis (EAE). In biochemical assays, the proCA showed a dose-response increase in R1 values in the presence of increasing concentrations of MMP-1, -9 or -12. In *ex-vivo* blood samples from EAE mice, the R1 values were significantly increased above healthy control values at the time of inflammatory peaks of the disease when MMPs are highly enriched in the bloodstream. Nanosizing the particle will allow testing of the pro-CA in *in vivo* situations.

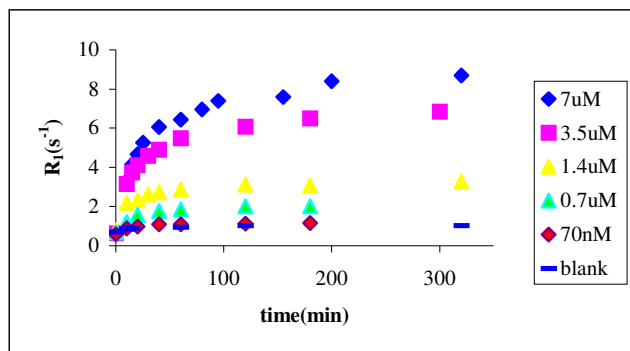


**Fig. 1: Biochemical assay: increasing R1 signal with increasing concentration of MMP12**

*In vivo* MRI modalities are being applied to visualise CNS lesions after induction of EAE in rats or mice. EAE models are characterised by inflammatory responses leading into demyelination and axonal loss within spinal cord and brain regions. To characterise the imaging signature of inflammatory lesions in a model of rat acute EAE, longitudinal T2 weighted and T1-Gadolinium signals have been measured over the disease course at the level of the pontine-cerebellar interface. As early as 6 days post-immunisation during the pre-clinical phase, leakage of the blood brain barrier (BBB) measured by increased Gadolinium signal in the pontine area can already be observed and further measures during disease development showed maximal increase in BBB leakage before the acute disease phase. This characterisation of MRI signals will allow testing of *in vivo* nanosized MMP-proCA during the next phases of the Work Package.

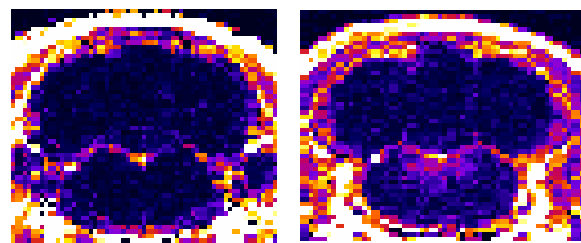


**Fig. 2: Enhancement maps of T1-Gadolinium in pontine regions of healthy (left) or EAE (right) acute EAE at day 6 post-immunisation**

### Conclusions from the first year

Current development of MMP-sensitive pro-CA as well as applications of MRI modalities to visualise and quantify CNS lesions in EAE models allows the progression towards the next steps of the work plan.

**WP leader:** RBM - Dr Beatrice Greco (beatrice.greco@merckserono.net)

**WP Participants:** UU, FOM, PHILIPS, RBM, BRACCO, CNRS, UNITO, GUERBET, PHILIPSD, IDT

## Work Package 9: Application to Cancer

### Objectives

- To study pharmacokinetics, tissue distribution, targeting efficiency, and therapeutic efficacy of the developed targeted nanomedicines in suitable animal models of cancer
- Therapeutic evaluation of MRI-guided drug delivery (triggered release) in animal models of cancer

### Work plan

Recruitment and training of staff is ongoing in all centres.

Preclinical cancer models demonstrated the efficacy of corticosteroids delivered by passive targeting of nanocarriers. Significant suppression of tumour progression was achieved and was characterised by immuno-histochemistry and by RNA expression profiling. The anti-tumour effect was attributed to suppression of the activity of Tumour Associated Macrophages resulting in attenuated expression of proangiogenic factors.

Multiparametric imaging methods are being developed for analysis of the tumour microenvironment, and for tracking delivery of nanoparticles targeted to tumour cells and to endothelial cells in the tumour neovasculature. Passive delivery of iron oxide particles

to tumours was followed by MRI. As early as one hour after administration of the contrast material, hypointensity in MRI was assigned to iron oxide sequestered by macrophages at the tumour rim. This approach can be utilised for following macrophage infiltration into tumours. The efficient uptake by Tumour Associated Macrophages has been further assessed by the use of paramagnetic liposomes.

Further studies are planned with new, emerging and approved drugs, along with introduction of targeted particles. Targeted delivery of anti-tumour agents will be challenged on resistant tumour cell lines in order to evaluate the ability to overcome multidrug resistance through alteration of the delivery mechanism. For that aim, new multidrug resistance models are being developed from cell lines with endogenously high drug sensitivity.

### Conclusions from the first year

Encouraging preclinical results were obtained for passive targeting of tumours with liposomal corticosteroids. The effect was attributed to suppression of Tumour Associated Macrophages and attenuation of tumour angiogenesis. MRI tools were developed for monitoring the recruitment of macrophages to tumours and for visualisation of tumour angiogenesis.

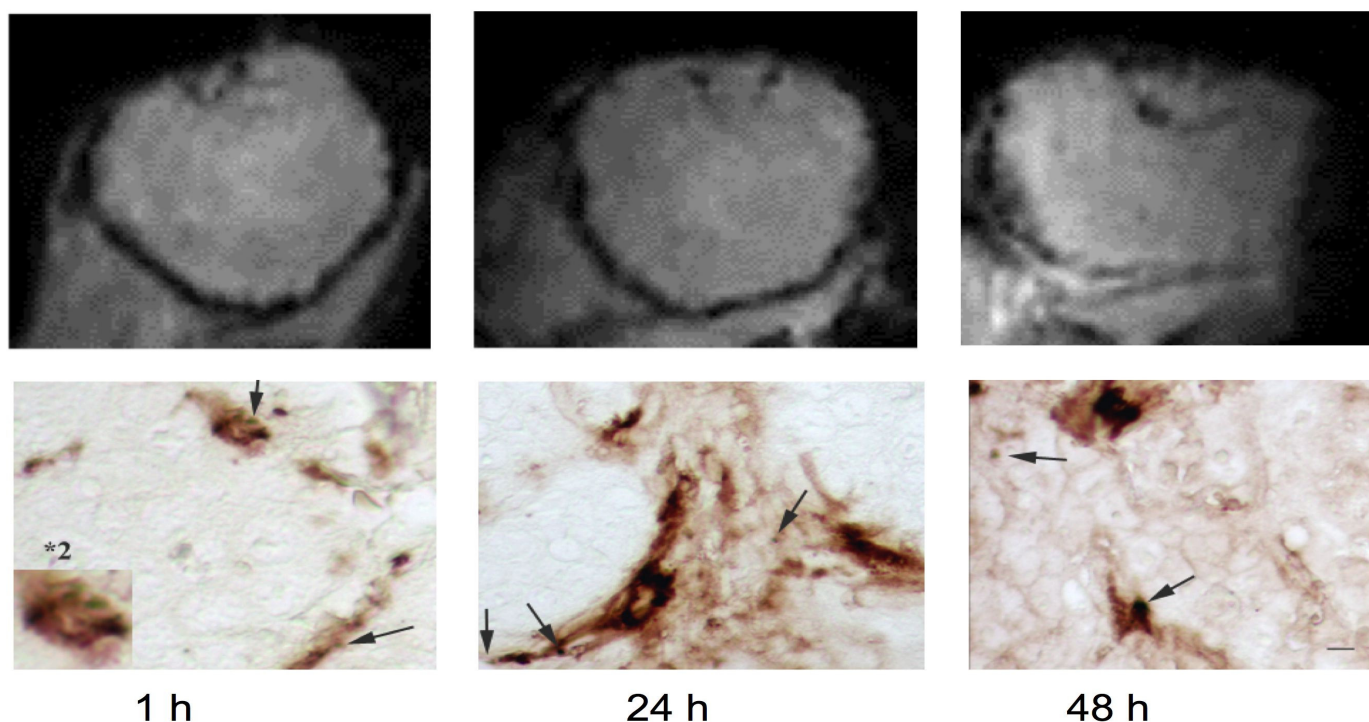


**Fig. 1: Passive targeting of P904 iron oxide particles to tumours. Top row) MRI follow up of iron oxide particles generating hypointensity in the rim of subcutaneous human ovarian carcinoma in nude mice. Bottom row) histological analysis demonstrating macrophage mediated delivery of the iron oxide particles to the tumour (macrophages stained in brown; arrows, Prussian blue stained iron oxide particles)**

**WP leader:** WEIZMANN - Prof. Dr Michal Neeman (michal.neeman@weizmann.ac.il)

**WP Participants:** UU, CHARITE, FOM, PCI, UL, ORGANON, TUE, MARBURG, BSP, PHILIPS, CNRS, WEIZMANN, UNITO, CSIC, GUERBET, CU, PHILIPSD, IDT

## Work Package 10: Preclinical Toxicology Commences in December 2008

### Objectives

- Assess the safety risks of selected prototype nanomedicines and provide guidance in the selection process of the final products
- Assess the toxicology aspects of the nanomedicines developed in MEDI TRANS in conjunction with NANOSAFE 2 and other EC projects

## Work Package 11: Industrial Exploitation Commences in December 2008

### Objectives

- To convert academic concepts into products
- To guide the prototype carrier systems through the predevelopment phase

## Work Package 12: Training

### Objectives

- To provide advanced drug delivery courses for partners and key stakeholders
- To develop the MEDI TRANS website as a platform for education and training, and to facilitate the exchange of MEDI TRANS' young scientists
- To provide access for MEDI TRANS' scientists to the training programme and events organised by the GALENOS-Network, and to create synergies
- To monitor and improve the efficiency of the training courses

### Work plan

#### Advanced drug delivery courses

The initial course on Drug Targeting Systems will be held on 23<sup>rd</sup> February 2008 at Saarland University. It is advertised on the MEDI TRANS website (see [www.meditrans-ip.net](http://www.meditrans-ip.net)). MEDI TRANS partners will be encouraged to participate in existing courses.

#### Internet-based training and a forum for scientific exchange

The MEDI TRANS' website is a forum for information exchange, and a tool for scientific exchange, training and education.

#### Training MEDI TRANS' and other young scientists

The exchange programme will involve secondment of young scientists to another partner's laboratory for several months, for intensive training. This will be organised *via* the MEDI TRANS' website. At the end of each secondment, the secondee will prepare an exchange report on "lessons learned".

#### Partner participation in the GALENOS-Network

All partners will be formally invited to join the GALENOS Network and to attend 10-day training courses on drug delivery, and on management. Graduate students will be encouraged to apply for a Euro PhD.

## Conclusions from the first year

The Drug Targeting Systems course has been postponed from year one of MEDI TRANS and will now be held on 23<sup>rd</sup> February 2008 at Saarland University.

The website has a page dedicated to training activities with sections on forthcoming, planned and recommended training courses, training course material, staff exchanges, PhD student networking and on the GALENOS Network.

The staff secondment programme and Galenos activities have been widely promoted.

## Work Package 13: Dissemination

### Objectives

- To demonstrate non-confidential technologies to MEDI TRANS' partners and other interested groups
- To develop, co-ordinate, and review appropriate dissemination strategies amongst the partners throughout the whole project duration
- To develop and maintain effective supporting channels of communication, including the dissemination contacts database, enquiry mechanisms and website
- To ensure effective dissemination of the project's results to interested stakeholders and the general public

### Work plan

#### Website development

The goal is for the website to be a powerful dissemination tool, and the interface between the project and the external community interested in research on targeted delivery of nanomedicines. A Contacts Database will be developed and maintained to support and assist in the co-ordination of all dissemination activities.

#### Project promotion

It is vital that the conclusions from MEDI TRANS are disseminated to as wide an audience as possible to increase the impact of the project's deliverables.

Glossy promotional leaflets will be distributed widely by partners at conferences, symposia, *etc.*, and to their normal business contacts.

## Conclusions from the first year

The MEDI TRANS website is available at [www.meditrans-ip.net](http://www.meditrans-ip.net). It has two main sections, the publicly accessible area and a members' area with password restricted access. The website contains a wealth of information on MEDI TRANS and enables interested users to contact us.

The database of contacts who will receive MEDI TRANS' disseminated results is in development.

Glossy promotional leaflets were produced in April and December 2007 and results have been disseminated.

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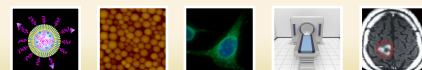
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