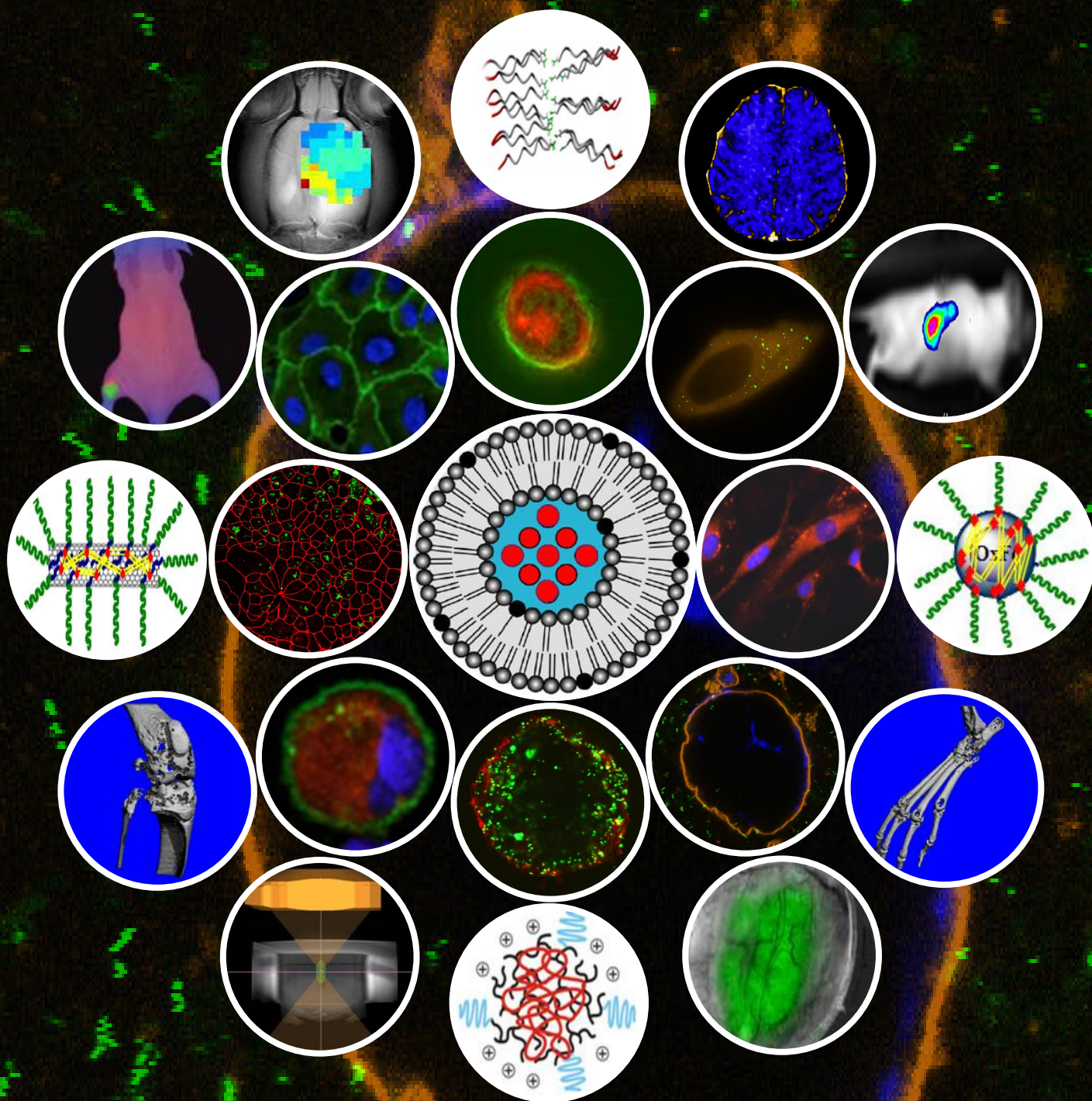


Targeted Delivery of Nanomedicine



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

Contract Number: NMP4-CT-2006-026668

Introduction

MEDI TRANS is a multidisciplinary Integrated Project dealing with targeted nanomedicines. The aim was for platform technologies to be developed with broad applicability to disease treatment, as exemplified by the choice for chronic inflammatory disorders (rheumatoid arthritis, Crohn's disease, multiple sclerosis), and cancer as target pathologies. Nanomedicines (based on carrier materials like polymeric and lipidic nanoparticles, nanotubes, and fullerenes) are being endowed with superior targeting and (triggerable) drug release properties. In parallel, MRI imaging probes are being designed that report on the localisation of the targeted nanomedicines, specific biomarkers, the drug release process and therapeutic outcome (imaging-guided drug delivery).

The consortium consists of 29 partners from 9 EU member states (including 1 new member state) and 3 associated states, and includes 12 industrial companies, 12 universities and 5 research institutes. The total budget is €14.8M with €10.7M as EC contribution and €4.1M as industrial support.

Project objective

To develop innovative targeted drug / imaging agent delivery, with controlled release and imaging guidance procedures for the detection of the underlying targeting / (triggered) drug release processes.

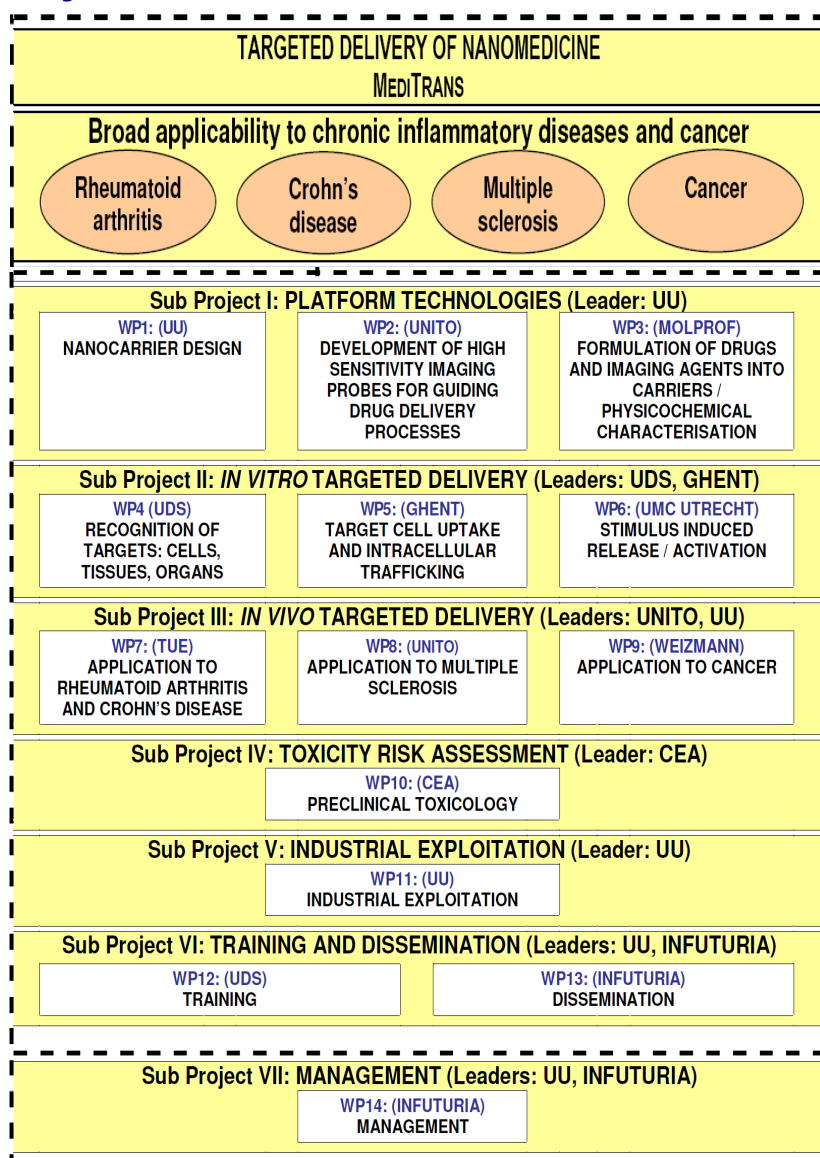
Specific challenges

- Promote entry of targeted nanomedicines into industrial exploitation and clinical proof-of-principle studies
- Develop non-invasive imaging procedures for monitoring of targeted drug delivery processes
- Demonstrate potential of emerging materials (*e.g.* fullerenes) for use as drug carrier materials

Impact

- Well-characterised targeted nanomedicines with broad applicability to disease treatment (rheumatoid arthritis, Crohn's disease, multiple sclerosis and cancer)
- Improved structural collaboration between industry and academia

Project structure



Images courtesy of:

- 1: Dr R. Schiffelers (UU)
- 2: Prof. Dr C.-M. Lehr (UDS)
- 3: Dr K. Fischer (BSP)
- 4: www.istockphoto.com
- 5: MRI brain image: Dousset *et al.* (2006). *American Journal of NeuroRadiology* 27: 1000-1005.

Front cover images

Background image: GHENT
A - 1 o'clock outer ring: PHILIPSD
B - 2 o'clock outer ring: TUE
C - 3 o'clock outer ring: CEA
D - 4 o'clock outer ring: TUE
E - 5 o'clock outer ring: WEIZMANN
F - 6 o'clock outer ring: MARBURG
G - 7 o'clock outer ring: PHILIPS / UMC UTRECHT
Planning for *in vitro* heating of fluorescent thermosensitive liposomes for tumour demarcation (developed by UMC UTRECHT) on the Philips Sonalleve MR guided HIFU system at PHILIPS.
H - 8 o'clock outer ring: TUE
I - 9 o'clock outer ring: CEA
J - 10 o'clock outer ring: WEIZMANN
K - 11 o'clock outer ring: CSIC
L - 12 o'clock outer ring: UU
M - 1-2 o'clock inner ring: GHENT
N - 3 o'clock inner ring: TUE
O - 4-5 o'clock inner ring: GHENT
P - 6 o'clock inner ring: UDS
Q - 7-8 o'clock inner ring: TUE
R - 9 o'clock inner ring: UDS
S - 10-11 o'clock inner ring: TUE
T - 12 o'clock inner ring: UNITO
U - central image: PHILIPS



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

Results and conclusions

The primary aim of Work Package 1 ("Nanocarrier Design") was to identify materials suitable for serving as drug delivery devices. Hereto, it on the one hand dealt with the synthesis and characterisation of *Emerging Materials*, like fullerenes and nanotubes, as well as, on the other hand, with the evaluation and optimisation of already existing *Candidate Materials*, like polyplexes, polymeric micelles and iron oxide nanoparticles.

Regarding the *Emerging Materials*, CEA, BRACCO and UU have in the past four years synthesized several different batches of fullerenes and nanotubes. In addition, nanotubes were ultrasound-shortened to sizes well below 500 nm (Figure 1A-C), and the surfaces of these carbon-based carrier materials were functionalised with carboxyl, hydroxyl and amine groups. Furthermore, nanotubes and fullerenes were surface-modified with a PEG-based hydrophilic stealth coating, and using both budesonide and siRNA, proof-of-principle was provided for drug loading and drug delivery. Finally, a large amount of data has been obtained on the cellular uptake and the *in vitro* cytotoxicity of fullerenes and nanotubes, showing that the particles are effectively taken up by macrophages (Figure 1D) and endothelial cells (Figure 1E), and that they are relatively well-tolerated at concentrations up to 1

mg/ml (*i.e.* the maximal concentration that could be reproducibly dispersed).

Concerning the *Candidate Materials*, except for Task 1.3.3. (which dealt with molecularly imprinted particles and which was halted in May 2009 because of lack target-specific re-binding), significant progress was made in all other project lines. Small-sized plasmid DNA-containing polyplexes were successfully generated, as were polyplexes containing nuclear localisation sequences, but both systems failed to effectively deliver DNA into nuclei (Figure 1F). For siRNA delivery, polyplexes, dendrimers, PLGA-based nanoparticles and nanogels were synthesized by, and regarding the latter, GHENT has shown that both PEGylated and unPEGylated nanogels are effectively taken up by cancer cells (Figure 1G). Concerning micelles, iron oxide nanoparticles and iron oxide-containing micelles (Figure 1H), UU and MAGFORCE have demonstrated that these systems can be effectively loaded with drugs, and that they can be modified (*i.e.* core-crosslinked and PEGylated) to circulate for prolonged periods of time. In addition to this, a library of amphiphilic amino acid-based peptides was generated (Figure 1I), and it was shown that several of these peptides form 50-100 nm-sized spherical particles, which can be loaded with model drugs such as calcein. Finally, various different types of stimuli-sensitive liposomes were developed, including *e.g.* systems which respond to hyperthermia and which above the transition temperature (T_m) of the lipids, effectively release encapsulated therapeutic agents and imaging probes (Figure 1J).

All *Candidate Materials* for which particularly promising results were obtained, *i.e.* siRNA-containing nanoparticles, polymeric micelles, iron oxide nanoparticles and temperature-sensitive liposomes, were forwarded to other Work Packages for more detailed *in vitro* and/or *in vivo* evaluation.

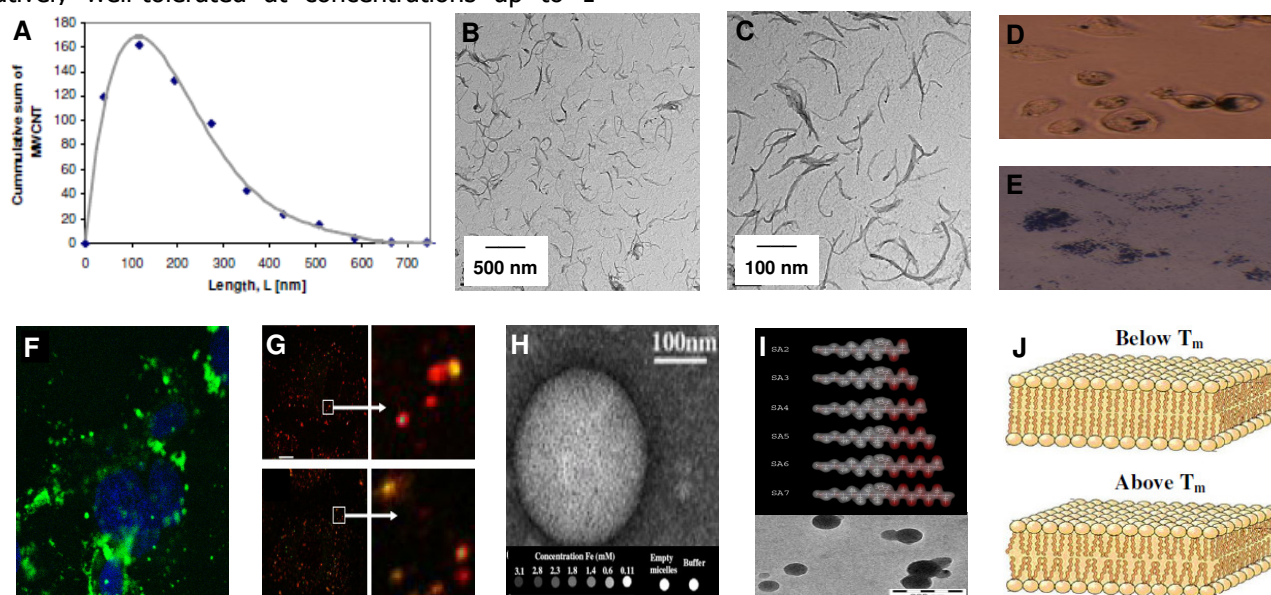


Figure 1: Overview of some of the nanocarrier systems developed as part of WP1. See text for details

WP1 Leader: UU - Prof. Dr Wim Hennink (w.e.hennink@uu.nl)

WP1 Participants: UU, CEA, MAGFORCE, GHENT, MARBURG, BRACCO, UNITO, CU, RUG

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

Results and conclusions

Task 2.1 - Highly sensitive Gd(III)-based agents

During the entire project UNITO and BRACCO synthesized several Gd(III) complexes endowed with high relaxivity. Among them, it is worth mentioning: i) a DTPA-like complex bearing a central phosphonic moiety and poly-hydroxyl branches, ii) a dimeric neutral macrocyclic chelate as a promising probe to be encapsulated in vesicular nanomedicine, iii) an amphiphilic Gd-AAZTA complex able to be incorporated in soft nanomaterials, and iv) paramagnetic liposomes encapsulating a clinically approved Gd(III) agent or incorporated amphiphilic Gd(III) complexes (also important contribution from TUE).

UNED has developed single-walled carbon nanotubes that displayed ferromagnetic behaviour and showed interesting MRI properties as contrast agents for diffusion weighted experiments.

Task 2.2 - Highly sensitive Chemical Exchange Saturation Transfer (CEST) agents

Highly-sensitive paramagnetic liposome-based CEST agents (LipoCEST) endowed with highly shifted intraliposomal water protons were developed by UNITO in collaboration with BRACCO. 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. The nanovesicles 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.

PHILIPS and PHILIPSD carried out work aimed at developing temperature-sensitive LipoCESTs for image-guided drug delivery.

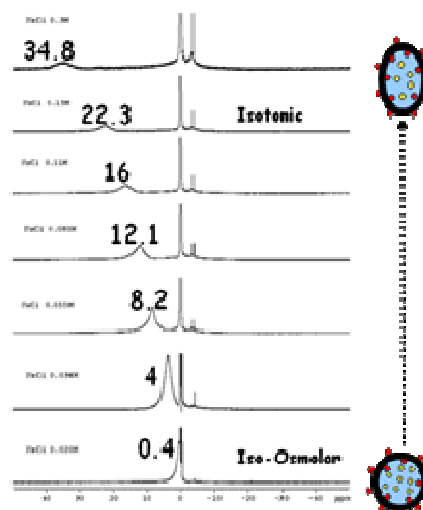


Figure 1: See text for details

These experiments showed that CEST contrast can be used to visualise the release of the liposomal content triggered by heating.

Task 2.3 - Novel iron-oxide based probes

Carbon-coated iron oxide particles (C@OxFe) produced by laser pyrolysis at CEA have been optimised as potential imaging probes for biological applications.

For such applications, a post-synthesis process of C@OxFe aggregates has been developed which afforded small particle clusters of *ca* 20-100 nm. The carbon-coated iron-oxide particles were successfully dispersed in water using Lipo-PEG2000 ligands. Relaxometric properties of these novel biocompatible particles were verified by GUERBET. Classical *r*₁ and *r*₂ values for such magnetic particles were measured.

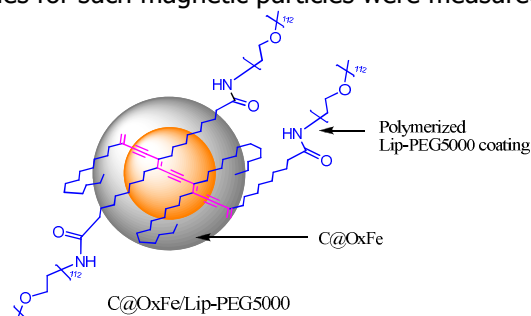


Figure 2: See text for details

Task 2.4 - Optical imaging probes

UNITO investigated the intracellular trafficking of fluorescent dyes with the aim of visualising, by optical tools, drug delivery and release processes. Optical visualisation of the intranuclear localisation of Doxil® and endosomal escape triggered by PCI technology were achieved. Dual optical/MRI probes were developed by TUE. In particular, successful work has been done with the aim of visualising collagenase, a biomarker of vulnerable atherosclerotic plaques.

WP2 Leader: UNITO - Prof. Dr Silvio Aime (silvio.aime@unito.it)

WP2 Participants: CEA, MAGFORCE, TUE, PHILIPS, BRACCO, UNITO, UNED, 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 MEDI TRANS

Results and conclusions

Emerging materials

- Carbon nanotubes have been successfully functionalised with budesonide, via a pyrene-PEG amphiphilic complex

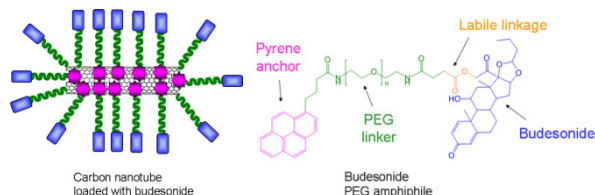


Figure 1: See text for details

Candidate materials

- Nanoparticles for intravenous siRNA delivery with encapsulation efficiencies >70% and sustained release of siRNA over several months have been achieved
- Physicochemical characterisation includes AFM, ToF-SIMS, XPS, zeta potential and particle size

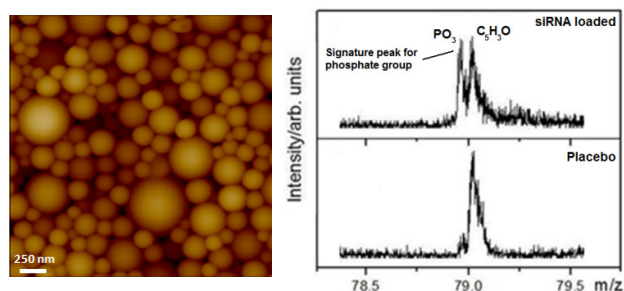


Figure 2: See text for details

- Dextran Nanogels $\approx$ 200 nm have been formulated with a high loading of siRNA (0.7 μ M). PEGylation does not affect the loading efficiency (below left)

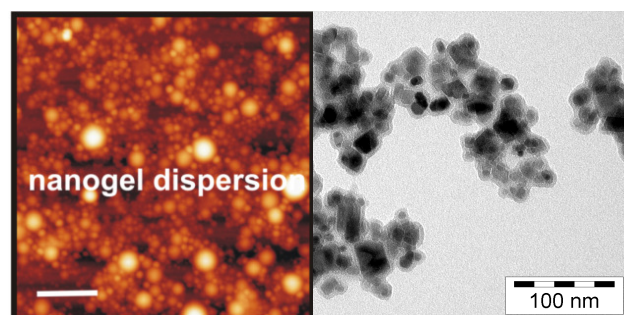


Figure 3: See text for details

- Iron-oxide nanoparticles (12-15 nm) have been loaded with Cisplatin and stealth coated with PEG (above right)

- Physicochemical characterisation includes IR, EDX, AFM, ToF-SIMS, XPS, XRD, zeta potential and particle size

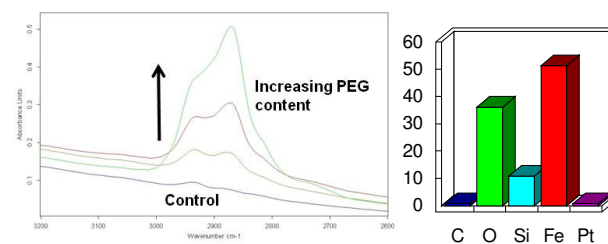


Figure 4: See text for details

- Polyplexes have been formulated for delivery of siRNA and DNA based on various polymers and targeting receptors

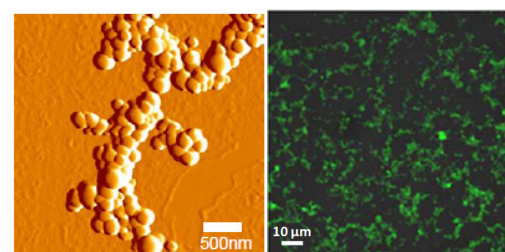


Figure 5: See text for details

Established materials

- Long *in vivo* circulating PEGylated liposomes, $\approx$ 100 nm in diameter, have been formulated with dexamethasone phosphate
- Liposomes have also been formulated with targeting ligands and pH responsive channel proteins, showing triggered release of a model compound

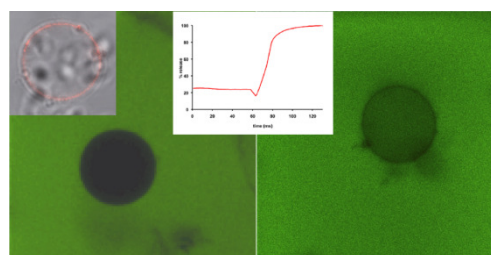


Figure 6: See text for details (image from Dudia, A. (2007) Nanofabricated Biohybrid Structures for Controlled Drug Delivery. PhD Thesis, University of Twente, Netherlands, 156p. In collaboration with Dr. Kocer's group.)

- Liposomes have been formulated containing Prednisolone and incorporating a Gd(III)-complex to act as MRI probes

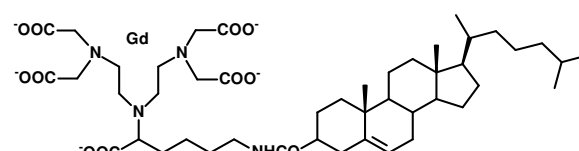


Figure 7: See text for details

- Monodisperse sub-200 nm PLGA nanoparticles were formulated with budesonide encapsulation efficiencies > 80%

WP3 Leader: MOLPROF - Dr Andrew Parker (aparker@molprofiles.co.uk)

WP3 Participants: UU, MAGFORCE, FOM, GHENT, ORGANON, MOLPROF, UDS, MARBURG, BSP, PHILIPS, MSSA, UNITO, CU, PHILIPSD, RUG



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)

Results

Task 4.1 - Recognition of targets in cancer

PEGylated magnetite nanocarriers were evaluated for passive tumour targeting (MAGFORCE, CHARITE). Non PEGylated nanocarriers were compared to PEGylated iron oxide nanoparticles with high and low levels of PEGylation with regards to their time dependent uptake into mouse mammary carcinoma cells. Results show a remarkably higher uptake of low level PEGylated particles by the cells.

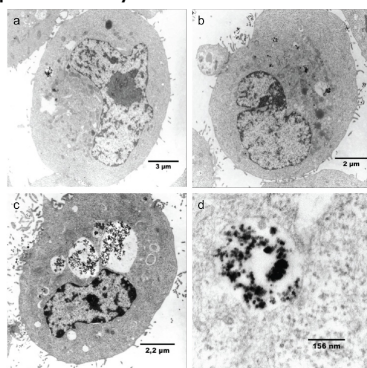


Figure 1: C3HRS1 cells at different time points of incubation with the PEGylated nanoparticles. a) after 10 min, b) after 30 min, c) after 24 hrs, d) the magnification of a vesicle after 24 hrs incubation time

Actively targeted liposomal nanocarriers with different targeting strategies were compared *in vitro*. Single targeted liposomes decorated with RGD peptide or Anginex ligand were compared to dually targeted formulations carrying both of these ligands at different densities (UU, WEIZMANN).

Results show that simultaneous targeting of both ligands improves the uptake of liposomes by activated endothelial cells compared to single-targeting and that dual targeting provides synergistic targeting effects in particular at high ligand densities.

Task 4.2 - Recognition of targets in CD

A 3D *in vitro* model of inflamed colonic mucosa consisting of enterocytes, dendritic cells and macrophages was established at UDS.

If stimulated by pro-inflammatory cytokine IL-1 β the model mimics pathophysiological changes of the diseased intestinal mucosa such as loss of barrier function and release of pro-inflammatory markers.

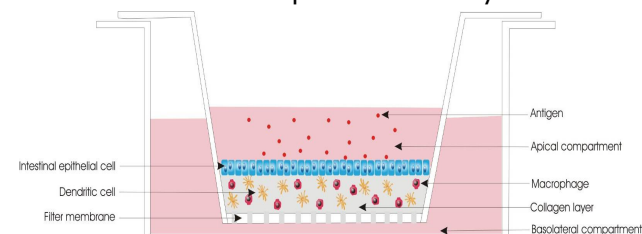


Figure 2: 3D *in vitro* model of inflamed colonic mucosa

The novel 3D *in vitro* model was successfully used to test and evaluate various MEDI TRANS budesonide and dexamethasone formulations for the therapy of IBD *in vitro*.

Task 4.3 - Recognition of targets in RA

Two different *in vitro* assays to predict extravasation ability of stealth formulations were developed in the course of the project. Uds quantified macrophage uptake of PEGylated and non-PEGylated formulations using fluorescent activated cell sorting. UU correlated *in vivo* circulation times of PEG 2000 and PEG 5000 decorated liposomes to plasma protein binding to the liposome surface using surface plasmon resonance technology.

Task 4.4 - Recognition of targets in MS

An *in vitro* model of the blood brain barrier with excellent barrier function and good transport properties for marker substrates based on a non-contact co-culture of porcine brain endothelial cells with astrocytes was successfully established (ACROSS).

Conclusions

All of the objectives of WP4 were met, at least in part, in the course of the project. While some approaches such as a diseased blood brain barrier model or active targeting of ICAM-1 in MS could not be realised, strong innovations were still made in WP4 and the *in vitro* testing of MEDI TRANS nanomedicines strongly contributed to their success and further exploitation.

In particular active and passive targeting concepts in cancer were investigated. Active targeting of liposomes for both drug delivery and MRI imaging to tumour vasculature was optimised.

Furthermore, sophisticated *in vitro* models such as the co-culture model of the inflamed intestinal mucosa, the healthy blood brain barrier model, and sophisticated assay principles such as the SPR based assay to predict extravasation ability of nanocarriers were developed.

WP4 Leader: UDS - Prof. Dr Claus-Michael Lehr (lehr@mx.uni-saarland.de)

WP4 Participants: UU, MAGFORCE, CHARITE, CSEM, UDS, ACROSS, 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 synthesize nanoparticles that can be used for light-triggered intracellular delivery of nucleic acids

Results and conclusions

In the first year, loading of siRNA in dextran based microgels was demonstrated. The gene silencing activity of these microgels with siRNA targeted against GFP was shown.

In the second year, siRNA was loaded in dextran based nanogels (typically 200 nm). These nanogels are efficiently taken up by cells and result in a biological activity that can be prolonged over time. Furthermore, the biological activity can be enhanced using photochemical internalisation (PCI).

In the third year, the nanogels were modified with a PEG-coating. Using SPT, PEGylation was shown to have a beneficial effect on nanogel aggregation in full human blood (Figure 1).

In the fourth year, several new methods were developed to study the behaviour of nanoparticles in complex extracellular and intracellular environments. Using both chemical and molecular inhibitors of specific endocytic pathways, the internalisation of nanoparticles by target cells was investigated. The results obtained from these studies can be used to improve the efficacy of nanoparticle formulations (Figure 2).

Current activities focus on the development of new methods for studying intracellular trafficking. In addition, the PEGylated nanogels are being modified with active targeting ligands and several nanoparticles for light-triggered intracellular delivery are evaluated for use *in vivo*.

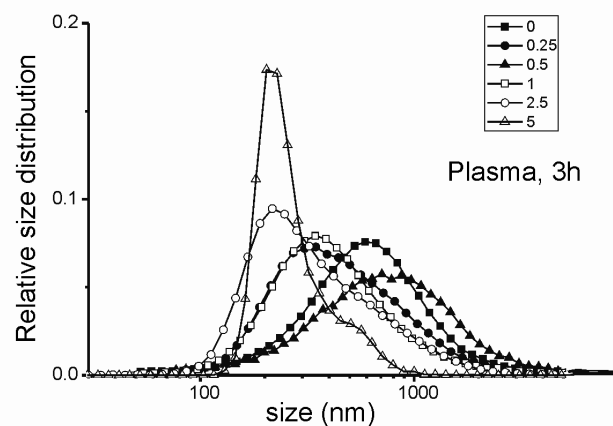


Figure 1: Single particle tracking was used to obtain relative size distributions of negatively charged nanogels after incubation with citrated plasma at 37°C. PEGylation of the nanogels was required to prevent their aggregation in human plasma (90%)

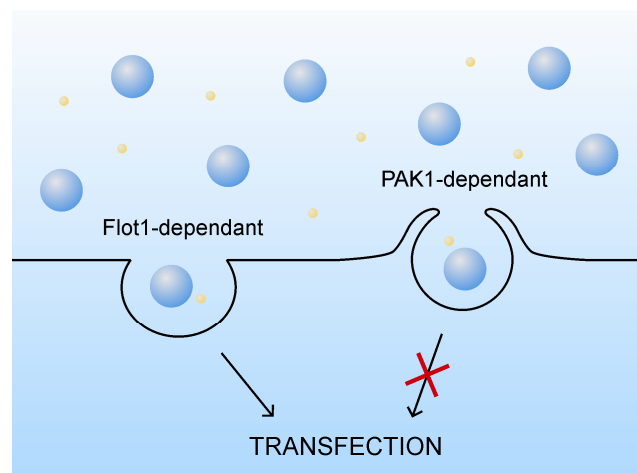


Figure 2: Studying nanoparticle internalisation using chemical and molecular inhibitors can lead to the design of nanoparticles with improved transfection efficacy

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

Results and conclusions

WP6 aims to explore ways to trigger the release of drugs from nanocarriers localised within the pathological region by means of external stimuli ("release on command") or by exploiting specific physicochemical characteristics of the pathophysiological microenvironment (internal stimuli).

Several nanocarriers were developed:

- Amino-functionalised $\text{FeO}_x\text{-SiO}_2$ particles that can induce local heating or can release drugs like cisplatin by using external magnetic fields (MAGFORCE)
- Nile red loaded polymeric micelles for image guided triggered release with High-intensity focused ultrasound (HIFU)
- Thermosensitive liposomes that release their content at 42°C
- DSPC-based thermo-sensitive stealth liposomes that release their MR imaging agents by the use of ultrasound heating or acoustic pressure
- pH responsive liposomes that release their content in the endosomes of cells due to the lower pH of 5.5
- Liposome-based channel protein technology for triggerable drug release after light and pH stimuli
- Liposome formulation containing an amphiphilic lipopeptide acting as a matrix metallo proteinase (MMP) substrate and can release its content in the presence of this enzyme which is overexpressed in several diseases (*e.g.* melanoma)

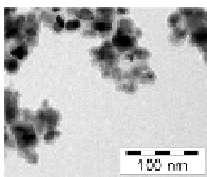


Figure 1: See text for details

PHILIPS and PHILIPSD developed dual 19F/1H-MRI CEST liposomal probes with contrast properties that are sensitive to temperature.

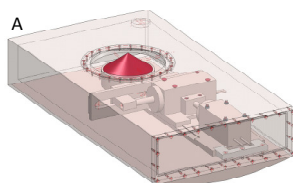


Figure 2: See text for details

An optimised magnetic resonance-guided HIFU system and an MR compatible HIFU system was designed and constructed, taking into account all requirements for performing ablation as well as hyperthermia experiments in small animals (UMC UTRECHT).

HIFU-sensitive liposomes were developed (UMC UTRECHT) and tested on a magnetic resonance-guided HIFU system (PHILIPS). The liposomes showed a release at 42°C.

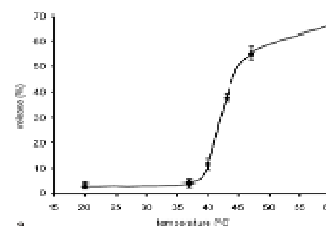


Figure 3: See text for details

A liposome with a remote controlled valve, a mechanosensitive channel of large conductance (MscL) has been engineered (RUG). Opening and closing of the channel could be controlled on command by both light and pH at the same time.

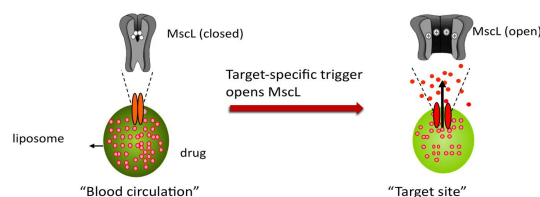
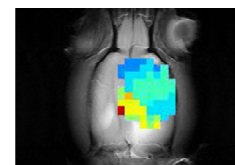


Figure 4: See text for details

An *in vivo* cerebral tumour model for the evaluation of extracellular pH effects in the delivery of



chemotherapeutic drugs from pH sensitive liposomes/particles was completed by CSIC and is now available for the MEDI TRANS researchers to test their pH sensitive particles.

A bifunctionalised carborane bearing a lipophilic chain and a MRI probe (Gadolinium; Gd) was synthesized by UNITO. The probe strongly binds to Low Density Lipoproteins (LDL) that are exploited to deliver the system to tumour cells.

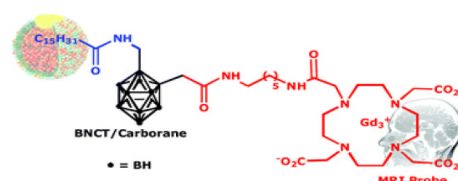


Figure 6: See text for details

Efficacy of the Boron/Gd/LDL adduct has been tested irradiating B16 tumour bearing mice by thermal neutrons 6 hours after administration and showed high efficacy resulting in arrested tumour growth.

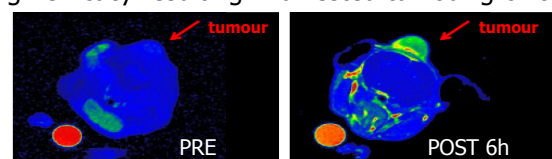


Figure 7: See text for details

WP6 Leader: UMC UTRECHT – Prof. Dr Peter Luijten (P.Luijten@umcutrecht.nl)

WP6 Participants: UU, CEA, MAGFORCE, PHILIPS, BRACCO, WEIZMANN, UNITO, CSIC, GUERBET, PHILIPSD, RUG, UMC UTRECHT



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 (RA) and Crohn's disease (CD)
- To evaluate and optimise targeted nanoparticles, loaded with imaging probes and drugs, for application in MRI guided imaging and therapy of inflammation

Results and conclusions

In vivo RA studies were performed at TUE jointly with UU. 10 male DBA1 mice (10 weeks of age) were used for the Collagen Induced Arthritis model. Disease severity was clinically scored by joint examination on a scale 0 – 2 and were scanned at different time points of the disease progress, and were treated with 10 mg/kg of saline as control, Dexamethasone (DXM) as free drug or Dexamethasone-PEG-liposomes (DXM-L) as nanocarrier drug. Animals were sacrificed in the end for histology. TUE developed a novel radio-frequency coil set-up for *in vivo* MRI studies on RA. MRI examination was performed on a 6.3 T scanner. The T1-weighted images (TR/TE=1000/10.2 ms) were recorded for different cross sections.

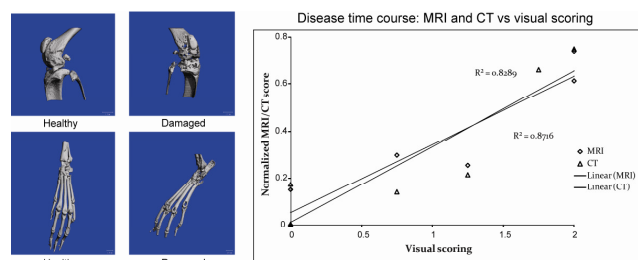


Figure 1: Left panel shows micro CT images of the paws and knees of mice, depicting a healthy (left) and a diseased animal (right). **Right panel** shows paw inflammation clinical scores vs total area of the MRI and CT images of the paws of mice

In this *ex vivo* study, MRI gave information about the soft tissues and fluid present in joints. This was complimented by CT, which gives information about the bone damage. The area of the sagittal MRI images and micro CT of the mouse paws, acquired at different stages of the disease progress, showed very good correlation with the disease progress score (Figure 1).

These encouraging preclinical results for monitoring the progress of the disease were used to evaluate anti-inflammatory therapy with MRI *in vivo*. A single dose of DXM did not result in a large decrease of paw inflammation scores (Figure 2). However, the same single dose encapsulated in PEG-liposomes resulted in a complete disappearance of paw inflammation at day 5 post-treatment, where after inflammation increased again. The data in this study demonstrate the profound anti-inflammatory activity of DXM-L, where the reduction of paw inflammation was rapid and the

therapeutic effect lasted more than a week. This indicates that the encapsulation of the drug in the nanocarrier system can strongly enhance its beneficial effect in RA.

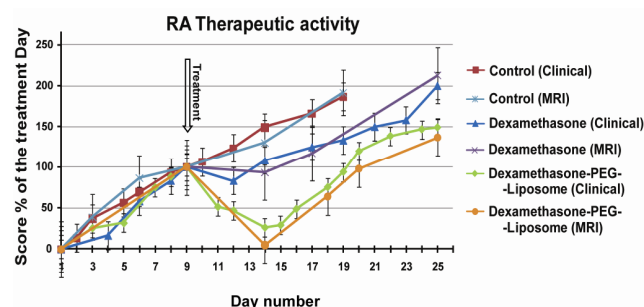


Figure 2: The graph shows paw inflammation clinical scores and total area of the MRI images of the paws of mice after single treatment. The score was set at 100 % at the day of treatment. Arrow indicates the day of treatment

CSIC developed a DSS animal model for CD studies. The 11 week old C57Black6 male mice received 2.5% (w/v) DSS in drinking water for 5 days, followed by two days on normal water. The treatment with DSS led to swelling of the ascending colon. A new protocol for 3D MRI visualisation of the progression of Gd (III) doped materials through the gastrointestinal tract in live mice was established. Isoflurane anesthetised adult mice received an intragastric administration of 0.4 mL Gd(III)DTPA and the passage of the contrast agent through the GI tract could be followed by MRI during the next four hours. Acquisition conditions were: TE/TR=10/450 ms, slice thickness = 1mm, matrix = 192x18x256 voxels.

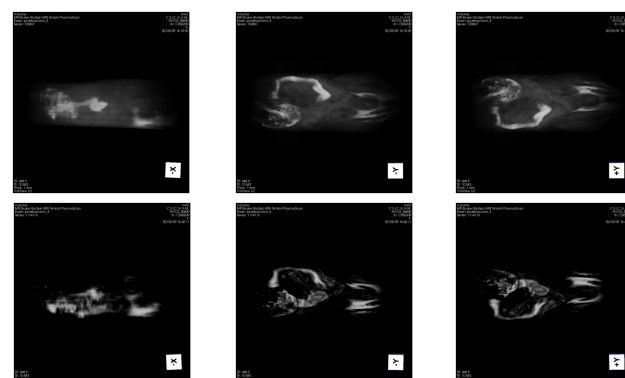


Figure 3: Snapshots extracted from 3D reconstructed images acquired 36 (upper row) and 72 (lower row) min after Gd-DTPA administration. The middle and right images are rotated 90° and 270° respectively from the initial orientation (left)

3D images were reconstructed with a maximal intensity projection algorithm. It was possible to observe the residual amount of contrast agent remaining in the stomach after the injection and how the different colonic cavities were filled sequentially, including the duodenum, jejunum, ileum and caecum. This MRI protocol is suited to study *in vivo* CD disease course.

WP7 Leader: TUE - Prof. Dr Klaas Nicolay (k.nicolay@tue.nl)

WP7 Participants: UU, FOM, TUE, UDS, BSP, PHILIPS, UNITO, CSIC, GUERBET, CU, PHILIPSD



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 matrix metalloproteinases (MMP)-inhibitors, through imaging-guided targeted and triggered delivery, in CNS lesions induced by MS-like pathology

Results and conclusions

Several Gd(III) based probes are under evaluation for the detection of MMP activity *in vivo* by MRI. All these probes are composed of a MMP cleavable peptide sequence (PLG**L*WAR) conjugated at the N-terminus with a Gd(III)DOTA chelate and at the C-terminus with a hydrophobic alkyl chain to improve the amphiphilic character of the molecule. The cleavage of the probe is expected to rescue a peptide fragment still bearing the Gd-DOTA complex, but having a more hydrophilic character, then different retention times within tissues (Figure 1). The MMP activity will then be evaluated by Dynamic Contrast Enhancement MRI, as the decay of the Gd(III) dependent signal enhancement is expected to be strongly influenced by the MMP dependent cleavage of the probe.

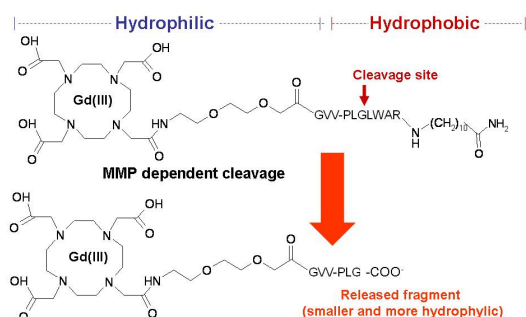


Figure 1: Structure of K11N, a MMP cleavable MRI probe

To prove this concept for the visualisation of MMP activity a subcutaneously induced tumour model has been considered. These tumours are larger than nerve lesions and MMP inhibitors (MMP-I) can be efficiently delivered into the region of interest by direct intratumour injection, thus minimising the issues linked to the pharmacokinetics of the drug. The MMP responsive contrast agent called K11N has been administered i.v. at 25 $\mu\text{mol/kg}$ to mice bearing a xenografted melanoma and T1-weighted spin echo images acquired over 4 hours. A second group of mice was injected (directly into the tumour) with the broad-spectrum MMP inhibitor Ilomastat at 30mg/kg prior to the administration of the CA. As expected, the signal enhancement decays slowly when tumours are treated

with Ilomastat (MMPs inhibited, the probe is not cleaved) and comparably faster when not treated (MMPs active, the Gd(III)-bearing peptide fragment released after MMP dependent cleavage is washed out from the tumour, Figure 2).

Having assessed that the K11N probe can detect the MMPs activity in the tumour model, attempts to obtain molecular images of MMPs in neurodegeneration have

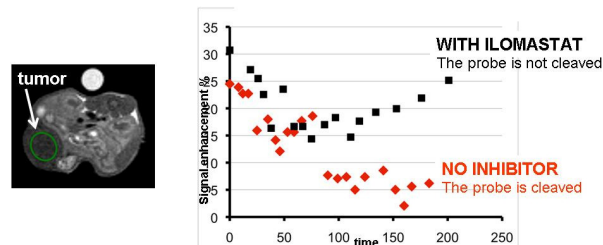


Figure 2: Decay of gadolinium enhancement in mice treated/not treated with the MMP-I Ilomastat

been carried out on the mouse nerve crush model of neuroinflammation. Compound K11N, administered i.v. via a catheter in the tail vein, was shown to accumulate selectively within the nerve lesion, and not in normal (non inflamed) nerves. By comparing the wash-in kinetics with those of extracellular and albumin binding CAs (Gd-HPDO3A and B22596, both from BRACCO), it has been shown that K11N in the blood is vehiculated by albumin and enters the nerve as the protein complex, in line with the low dissociation constant ($K_D = 50 \mu\text{M}$). After entering the nerve, K11N dissociates from albumin and interacts with the hydrophobic components of the ECM and fats.

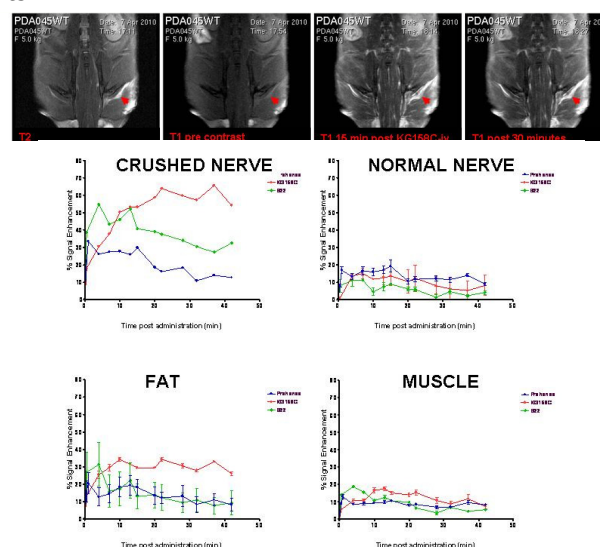


Figure 3: Gd enhancement as a function of time in several tissues. Red: K11N; Green: B22596 Blue: Prohance

However, the wash out kinetics in this model of neuroinflammation, as a function of the delivery of MMP-I, could not be evaluated because of severe adverse effects encountered with K11N at the dose level required. Actions to circumvent this problem are underway.

WP8 Leader: UNITO - Dr Giuseppe Digilio (giuseppe.digilio@unito.it)

WP8 Participants: UU, FOM, MSSA, UNITO



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

Results and conclusions

Core-crosslinked polymeric micelles with controlled release of covalently entrapped doxorubicin (Uu)

Doxorubicin (DOX) is clinically applied in cancer therapy, but its use is associated with dose limiting severe side effects. Core-crosslinked biodegradable polymeric micelles have shown prolonged circulation in the blood stream and enhanced tumour accumulation. A DOX methacrylamide derivative (DOX-MA) was covalently incorporated into the micellar core. The structure of the doxorubicin derivative is susceptible to pH-sensitive hydrolysis, enabling controlled release of the drug in acidic conditions. The entire drug payload was released within 24 hours incubation at pH 5 and 37 °C, whereas only around 5 % release was observed at pH 7.4. DOX micelles showed higher cytotoxicity in B16F10 and OVCAR-3 cells compared to DOX-MA, likely due to cellular uptake of the micelles via endocytosis and intracellular drug release in the acidic organelles. The micelles showed better antitumour activity than free DOX in mice bearing B16F10 melanoma carcinoma.

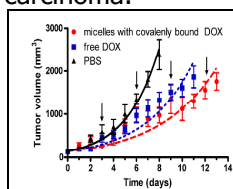


Figure 1: Treatment of mice bearing B16 melanoma with PBS, free doxorubicin (3 mg/kg), and micelles with covalently bound DOX (3 mg/kg). Arrows represent i.v. injections. Tumour volume ($P < 0.01$; F-test)

Pegylated spions for thermotherapy (CHARITE)

Treatment with an intratumoural thermotherapy using magnetic nanoparticles improved survival compared to conventional therapies in the treatment of recurrent glioblastoma multiforme. For a systemic treatment SPIONs should be stable against agglomeration under physiological conditions, and exhibit immunological stealthiness to avoid NP elimination from the blood circulation. These properties can be modulated by coating with polyethylene glycol (PEG).

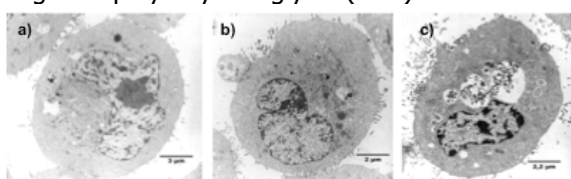


Figure 2: Time-dependent uptake of PEGylated NPs by C3H RS1 cells. a) Cell with PEGylated NPs after 10 min, b) after 30 min, c) after 24 h

Active targeting of doubly labelled liposomes and anti-inflammatory treatments in Oncology (TUE, WEIZMANN)

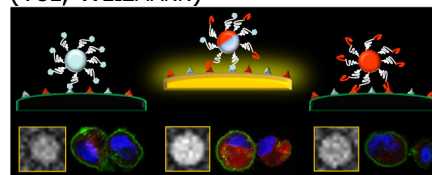


Figure 3: See text for details

Nanoparticles that functionalised with two angiogenesis-specific targeting ligands, an $\alpha v \beta 3$ integrin-specific and a galectin-1-specific peptide.

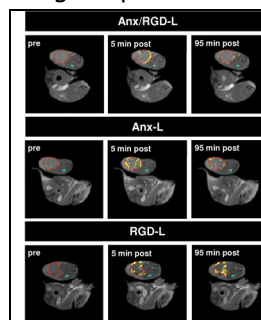


Figure 4: Optical techniques and MRI, showed that the dual-targeting approach produces synergistic targeting effects, causing a dramatically elevated uptake of nanoparticles into cultured endothelial cells as compared to single ligand targeting. T1-weighted MRI of liposome delivery to tumour

Changes in tumour vascular function after liposomal prednisolone phosphate treatment, as assessed with macromolecular DCE-MRI (TUE, UU, WEIZMANN)

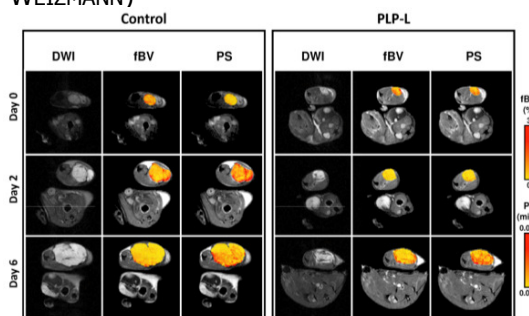


Figure 5: See text for details

Spectroscopic probes for the tumour extracellular environment (CSIC)

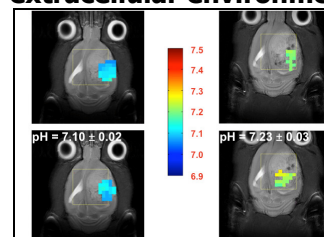
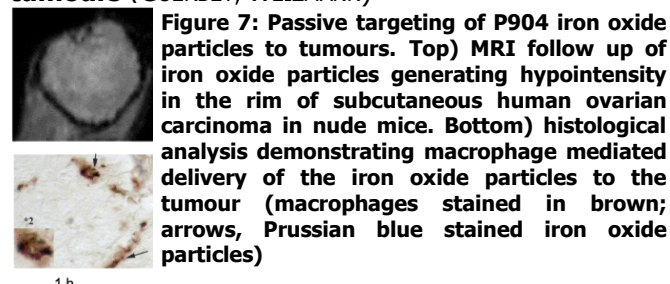


Figure 6: Changes in pH mapped by molecular imaging using MRI. Left) Rat C6 pH 7.10 ± 0.02 ; Right) Rat C6 bicarbonate pH 7.20 ± 0.01

Passive targeting of iron oxide particles to tumours (GUERBET, WEIZMANN)



WP9 Leader: WEIZMANN - Prof. Dr Michal Neeman (michal.neeman@weizmann.ac.il)

WP9 Participants: UU, RUG, CHARITE, FOM, PCI, GHENT, UL, ORGANON, TUE, MARBURG, BSP, PHILIPS, UMC UTRECHT, WEIZMANN, UNITO, CSIC, GUERBET, CU, PHILIPSD



Work Package 10: Preclinical Toxicology

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

Results and conclusions

WP10 aims to study the potential adverse effects of the selected nanomedicines, prior to clinical studies. The main work plan is based on pharmacology safety tests which include pharmacokinetic, biodistribution, and toxicology studies.

Four nanomedicines were selected:

- Two nanomedicines for the treatment of cancer: Liposomal Dexamethasone and Polymeric Micelles
- Two nanomedicines for imaging-guided drug delivery: Liposomal Gadolinium and Ultrasmall SuperParamagnetic Iron-Oxide (USPIO)

The four nanomedicines are currently being investigated and only preliminary data are available as of November 2010.

Polymeric micelles were loaded with an anti-cancer drug and injected into mice, and the animal behaviour was monitored (so-called Irwin test). This experiment indicated that micelles were well tolerated as no relevant effects were detected. Very recently, a maximum tolerated dose (MTD) study was performed in mice and a confirmation study is currently ongoing. The first results indicate that there is no acute toxicity up to rather high concentrations (85 mg/kg). Histopathological examination will be performed soon in order to confirm the absence of organ damage.

The same type of experiment was conducted with Liposomal Gadolinium leading to the same conclusion. There are no adverse effects associated with the liposomal formulation. In addition, biodistribution of Liposomal Gadolinium was also investigated. The *in vivo* phase is concluded but analytical determinations are ongoing.

The *in vitro* toxicity of Ultrasmall SuperParamagnetic Iron-Oxide nanoparticles (USPIO) was assessed by GUERBET, showing neither cytotoxic nor pro-inflammatory effects. It was also shown that the USPIOs do not activate the complement system. In parallel, systemic toxicity in rodent models was studied through haematological and histopathological examinations. Favourable data were obtained and no USPIO accumulation, other than in the expected organs, was detected. Finally, the lethal dose (LD) was determined to be 500 times higher than the clinical dose.

WP10 Leader: CEA - Dr Eric Doris (eric.doris@cea.fr)
WP10 Participants: CEA, UU, BRACCO, GUERBET



Work Package 11: Industrial Exploitation

Objectives

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

Results

The following nanomedicines have been selected:

1) Liposomal dexamethasone for treatment of cancer

Liposomal corticosteroids designed to achieve inhibition of cancer-related inflammation have become one of the most successful targeted nanomedicines within MEDI TRANS.

A manufacturing process for liposomal dexamethasone has been successfully set up. This new process allows production at a clinical scale (1 litre batches) and can be performed under GMP conditions. Also part of the manufacturing procedure is a sterilisation step and the sterile filling process. A full set of characterisation assays has been set up in order to be able to check and control drug and excipient identity, content and purity, particle characteristics, sterility and pyrogenicity.

2) Polymeric micelles for treatment of cancer

Core-crosslinked micelles represent a novel, highly attractive platform technology suitable for the targeted delivery of small molecules and/or imaging agents.

Synthesis routes to prepare materials (monomers, macroinitiator and resulting block copolymers) on a larger scale and more cost-effectively have been scouted, and yielded valuable insights. A device for the continuous formulation of drug-loaded micelles is under development. This is done in collaboration with analytical chemistry, process engineers and experts from the pharmaceutical industry to assure ease of implementation in a GMP process in the near future.

A freeze-thaw study of paclitaxel-loaded micelles indicated that this formulation is stable up to at least 3 cycles as measured by an identical particle size, polydispersity and drug release profile. Additional stability studies are currently in progress. Moreover, a method to freeze-dry drug-loaded micelles and to obtain a stable product is successfully developed.

A basic GLP system is set up and existing analytical methods are currently being recorded in protocols. This should ensure traceability of all raw materials and enable fast technology transfer to synthesis and formulation of drug-loaded micelles under GMP conditions.

3) Liposomal gadolinium for imaging-guided drug delivery

The research work carried out in MEDI TRANS has generated different types of Gd-loaded liposomes that

yield a marked contrast in the MR images of the regions in which they distribute and are therefore an innovative diagnostic tool.

A scalable preparation process of Gd-HPDO3A-loaded liposomes was designed following similar criteria and methodology used in the GMP-manufacturing process of the liposomal dexamethasone formulation. The Gd-loaded liposomes were prepared using a one step process where the sizing of the liposomes is obtained by a high pressure homogeniser. However, the whole process requires the elimination from the formulation of the excess of Gd-HPDO3A by tangential ultrafiltration and the corresponding recovery/recycling loop. The process optimisation was carried out on a lab scale up to 100 ml/batch.

A series of batches were prepared in order to evaluate the intra-batch reproducibility of the process, while a robustness study for the next technology transfer to an upper production scale is under evaluation. All the analytical methods needed for end product characterisation and quality control were validated according to GMP requirements.

Two preliminary stability studies at 4 °C and 25 °C are also on-going.

4) Ultrasmall superparamagnetic iron-oxide (USPIO) nanoparticles for imaging-guided drug delivery

P904 is a small nanoparticle with a low polydispersity and a relatively long half-life in the blood, thus allowing uptake of P904 mainly by activated macrophages that are involved in the physiopathological process of several diseases.

ICH accelerated and long-term stability studies have been performed on two P904 preclinical batches. The samples were packaged in a clear glass bottle, closed by a plastic stopper. No modification in appearance, pH, diameter or magnetic susceptibility was observed in both preclinical batches under accelerated conditions, at 40°C during 6 months or at 25°C during 12 months. The percentage of the two main impurities did not increase during these stability studies and was far below the specification limits.

To conclude, the colloidal solution of superparamagnetic iron oxide, P904, from both preclinical batches is stable during 6 months at 40°C/75% RH and 24 months at 25°C/60% RH.

Conclusions

Overall, significant and successful progress has been made in the late-stage preclinical development of the four selected nanomedicines. Consequently, these compounds will proceed further in the development chain towards clinical evaluation.

WP11 Leader: UU - Dr Cristianne Rijcken (c.j.f.rijcken@uu.nl)

WP11 Participants: UU, BRACCO, GUERBET



Work Package 12: Training

Objectives

- To provide advanced drug delivery courses for partners
- 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

Results

Advanced drug delivery courses

The initial course was on 'Drug Targeting Systems' and was held on 23rd February 2008 at UDS. More than 17 participants attended.

The second course was on 'MRI Technologies for Drug Delivery' and was held on 29th March 2009 at WEIZMANN. Approximately 45 participants attended.

The third course was on 'Targeting and Imaging' and was held on 22nd March 2010 at UDS. Between 100-150 participants attended.

The final course was titled 'MEDI TRANS PhD student oral presentation competition'. It was held on 30th October 2010 at Hotel Eden Roc, Sant Feliu de Guixols, Spain. There were about 30 attendees. Prizes were awarded to Broes Naeye, Bart Crielaard and Chris Oerlemans.

These four courses were advertised to MEDI TRANS partners and were actively promoted. In addition, MEDI TRANS partners participated in other existing courses.

Internet-based training and a forum for scientific exchange

Training pages of the public and members' areas of the MEDI TRANS website were prepared along with the MEDI TRANS forum for scientific exchange in the first year of the project. They were kept up-to-date throughout the project, by INFUTURIA, with the training material provided by the MEDI TRANS partners.

Training MEDI TRANS' and other young scientists

The exchange programme saw the secondment of young scientists to other partner's laboratories for intensive training. This programme was organised via the MEDI TRANS' website.

In the first year, the training sections of the website were developed, the staff exchange programme was promoted, names and contact details were collected, and staff exchange forms were completed and collated. This programme continued, as planned, throughout the duration of MEDI TRANS. In 2008 there were 4 exchanges, in 2009, there were 2, and in 2010 there were 5 exchanges. At the end of each exchange, each participant prepared exchange reports on 'lessons learned'.

Partner participation in the GALENOS-Network

All MEDI TRANS partners were formally invited to join the GALENOS Network and to attend 10-day training courses on drug delivery, and on management.

In addition to the seven MEDI TRANS partners who were members of the GALENOS network prior to the start of MEDI TRANS, TUE joined the GALENOS network during the project.

Euro PhD

The following MEDI TRANS participants gained their Euro PhD during the active phase of WP12:

- It is expected that at least one more MEDI TRANS PhD student will receive their Euro PhD in 2010
- Dr Verena Hengst (2008): Heinrich-Heine University Duesseldorf / DE (Home) and Utrecht University / NL (Host)
- Dr Manuela Banciu (2008): University of Cluj / RO (Home) and Utrecht University / NL (Host)
- Dr Marjan Fretz (2007): Utrecht University / NL (Home) and University of Cardiff / UK (Host)

Conclusions

All of the objectives of WP12 were met during the course of the project.

Four advanced drug delivery courses were provided for partners and key stakeholders and the effectiveness of the courses was monitored. All four courses were appreciated by the participants and were shown to be successful.

The MEDI TRANS website was developed as a platform for education and training, and to facilitate the exchange of MEDI TRANS' young scientists. The website training page had sections on forthcoming, planned and recommended training courses, training course material, staff exchanges, PhD student networking and on the GALENOS Network.

WP12 provided access for MEDI TRANS' scientists to the training programme and events organised by the GALENOS-Network. Synergies were created. Eleven MEDI TRANS' staff went on exchange and GALENOS activities were widely promoted.

In summary, relevant, up-to-date training was provided to MEDI TRANS partners implemented via courses, website-based information, exchange to other partners labs, and via the GALENOS Network. This enabled MEDI TRANS employees to work as effectively as possible and to pass on the new skills they learned to others.

WP12 Leader: UDS - Prof. Dr Claus-Michael Lehr (lehr@mx.uni-saarland.de)

WP12 Participants: UU, INFUTURIA, UDS, UNITO, UMC UTRECHT



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

Results

Demonstration of non-confidential technologies

Demonstrations of, and seminars on, MEDI TRANS' non-confidential technologies, structured in a manner suitable for MEDI TRANS' scientists, SMEs, and other interested stakeholders, took place at partner facilities in 2010:

- MOLPROF held a webinar on 8 December 2010 at 16.00pm CET
- UDS produced two educational movies on the setup and handling of the 3D Crohn's model and on the extravasation assay
- UDS organised a public demonstration course on 'Innovative *in vitro* models and assays in MEDI TRANS' on 27 March 2010
- PHILIPS/PHILIPSD are planning a demonstration video based on the concept of temperature-sensitive liposomes for MRI guided delivery
- GUERBET demonstrated MRI guided drug/contrast media (notably P904) delivery at an Imaging Conference for French physicians and radiologists
- BRACCO held a demonstration workshop on imaging diagnostics and nanotechnologies on 14 July 2010

Website development

The project website was put online in Month 5 of the project. The project website has two main sections, the public and members' areas. The members' area has password restricted access and is only for use by MEDI TRANS members and by the EC. The website text was prepared by UU with contributions from selected MEDI TRANS partners. The project's members' forum was also password protected. It contained forums for each WP, for end users, and for the PSC. The forum was discontinued in 2010 as partners preferred to communicate via e-mail. Website content was reviewed and approved by the MEDI TRANS consortium. The website was maintained, developed and updated throughout the project. By the end of November 2010, the website had >58,377 visitors, with >511,999 hits and with >172,724 page views. There were >51.3 visitors per day on average, with the USA and Germany being the most active countries. Of the MEDI TRANS partners' websites, MARBURG provided most referrals.

Web pages added later in the project included: News, Useful links, Public awareness, MEDI TRANS publications, and Partner website links. In 2009, a web based presentation was added.

Also, a confidential contacts database was prepared and was used to invite key stakeholders to the technology transfer workshops and to the dissemination event.

Project promotion

The conclusions from MEDI TRANS were disseminated to as wide an audience as possible to increase the impact of the project's deliverables.

Five sets of MEDI TRANS promotional leaflets were prepared and were distributed widely by partners at conferences, symposia, *etc.*, and to their normal business contacts. The leaflets, which can be downloaded from the website, provide an annual overview of the projects' objectives, work plan, and of the results achieved.

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 MEDI TRANS to the research community. It is available to download from the website.

Other project promotional material, for example, the MEDI TRANS promotional slides, was also designed.

Technology transfer workshops and the dissemination event

The first technology transfer workshop was combined with the EuroNanoMedicine Conference (Bled, Slovenia, 28th–30th September 2009). It was jointly organised by the three FP6 EC Integrated Projects, MEDI TRANS, NANOEAR and NANOBIOPHARMACEUTICS. MEDI TRANS partners contributed to the organisational committees, to conference dissemination activities, and to session chairing. The 50 MEDI TRANS attendees contributed 14 lectures and 12 posters. This well attended conference transferred interesting results to key stakeholders.

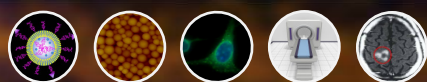
The second technology transfer workshop and the dissemination event were combined with the ESF 2010 Conference, Nanomedicine: Reality Now and Soon (Sant Feliu de Guixols, Spain, 23rd–28th October 2010). MEDI TRANS partners contributed to 'Session 10: Targeted Nanomedicine and the EC' and to the Poster Sessions. The 25 MEDI TRANS attendees contributed 10 lectures and 13 posters. The conference transferred and disseminated interesting results and was well attended.

Conclusions

All of the objectives of WP13 were met during the course of the project. Non-confidential technologies were demonstrated to MEDI TRANS' partners and other interested groups. Effective supporting channels of communication, including the dissemination contacts database, enquiry mechanisms and website were developed and maintained. The project's results were disseminated to interested stakeholders and to the general public using appropriate means.

WP13 Leader: INFUTURIA - Mr Seymour Kurtz (seymourkurtz@aol.com)

WP13 Participants: UU, INFUTURIA, CEA, CHARITE, FOM, RUG



CONSORTIUM



Universiteit Utrecht

Netherlands
Prof. Dr Gert Storm (Co-ordinator)
www.pharm.uu.nl/pharmaceutics/



Commissariat À L'Énergie Atomique

France
Dr Frédéric Schuster
www cea.fr



Magforce Nanotechnologies AG

Germany
Dr Andreas Jordan
www.magforce.com



Charité Universitätsmedizin Berlin

Germany
Prof. Dr Peter Wust
www.charite.de



FOM

Netherlands
Prof. Dr Ron M. A. 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.schering-plough.com



Technische Universiteit Eindhoven

Netherlands
Prof. Dr Klaas Nicolay
www.tue.nl



molecular profiles
experts in analysis

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/de/fakultaeten/fak8/f82/lehr/



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



Bracco Imaging SpA

Italy
Dr Alessandro Maiocchi
www.bracco.com/Bracco/home.htm



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



Universidad Nacional de Educacion a Distancia

Spain
Prof. Dr Paloma Ballesteros García
www.uned.es



Infutura Group AG (Project Managers)

Switzerland
Mr Seymour Kurtz / Dr William Dawson
[www.infuturiagroup.com /](http://www.infuturiagroup.com/) www.infutura.eu



Rijksuniversiteit Groningen

Netherlands
Dr Armagan Kocer
www.rug.nl



Universitair Medisch Centrum Utrecht

Netherlands
Prof. Dr Peter Luijten
www.umcutrecht.nl



European Commission Project Officer
Dr George Kirmizidis

Contact us via the **Contact Us** page of the website:
www.meditrans-ip.net

Former consortium members:



Instituto di R.B.M. SpA (It)

Dr Beatrice Greco www.company.merckserono.net

Integrated DNA Technologies, BVBA (Be)

Dr Brian Sproat <http://eu.idtdna.com>



New consortium member:

Merck-Serono S.A. (Ch)

Dr Michel Dreano www.company.merckserono.net

