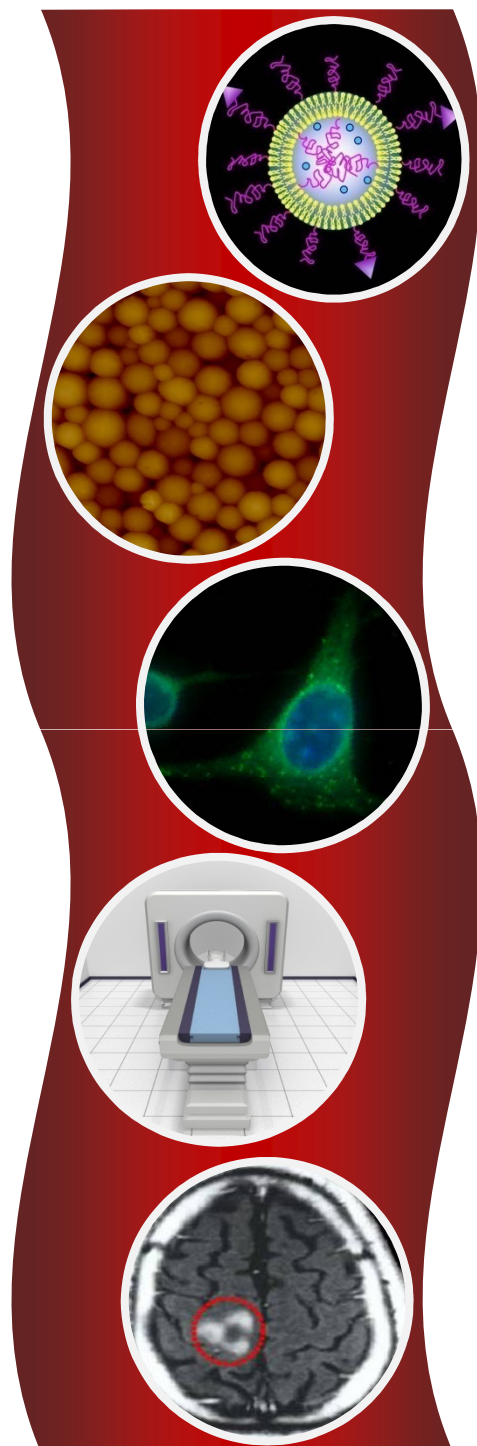




Targeted Delivery of Nanomedicine

Web-based presentation

www.meditrans-ip.net

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

Contract Number: NMP4-CT-2006-026668



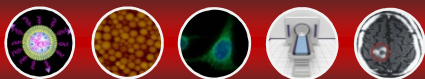
Targeted Delivery of Nanomedicine



Co-ordinator
Utrecht University
Prof. Dr. Gert Storm

EU funding: 11 million Euro
Duration: 48 months
Start date: 1st January 2007
End date: 31st December 2010

Project's website:
www.meditrans-ip.net



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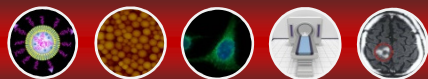
Targeted Delivery of Nanomedicine



Summary

MEDITRANS represents a multidisciplinary Integrated Project dealing with targeted nanomedicines. Platform technologies will 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) will be endowed with superior targeting and (triggerable) drug release properties. In parallel, MRI imaging probes will be 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 30 partners from 9 EU member states (including 1 new member state) and 3 associated states, and includes 13 industrial companies, 12 universities and 5 research institutes. The total budget is €15.8M, with €11M from the EC and €4.8M from MEDITRANS' industrial partners.



Targeted Delivery of Nanomedicine



Project objective

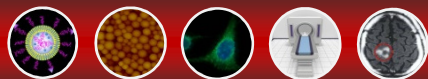
- To develop innovative targeted drug / imaging agent delivery, with controlled release and imaging guidance procedures for the detection of the underlying targeting / (triggered) drug release processes

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

Expected impact

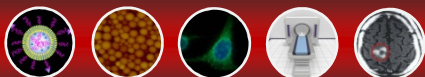
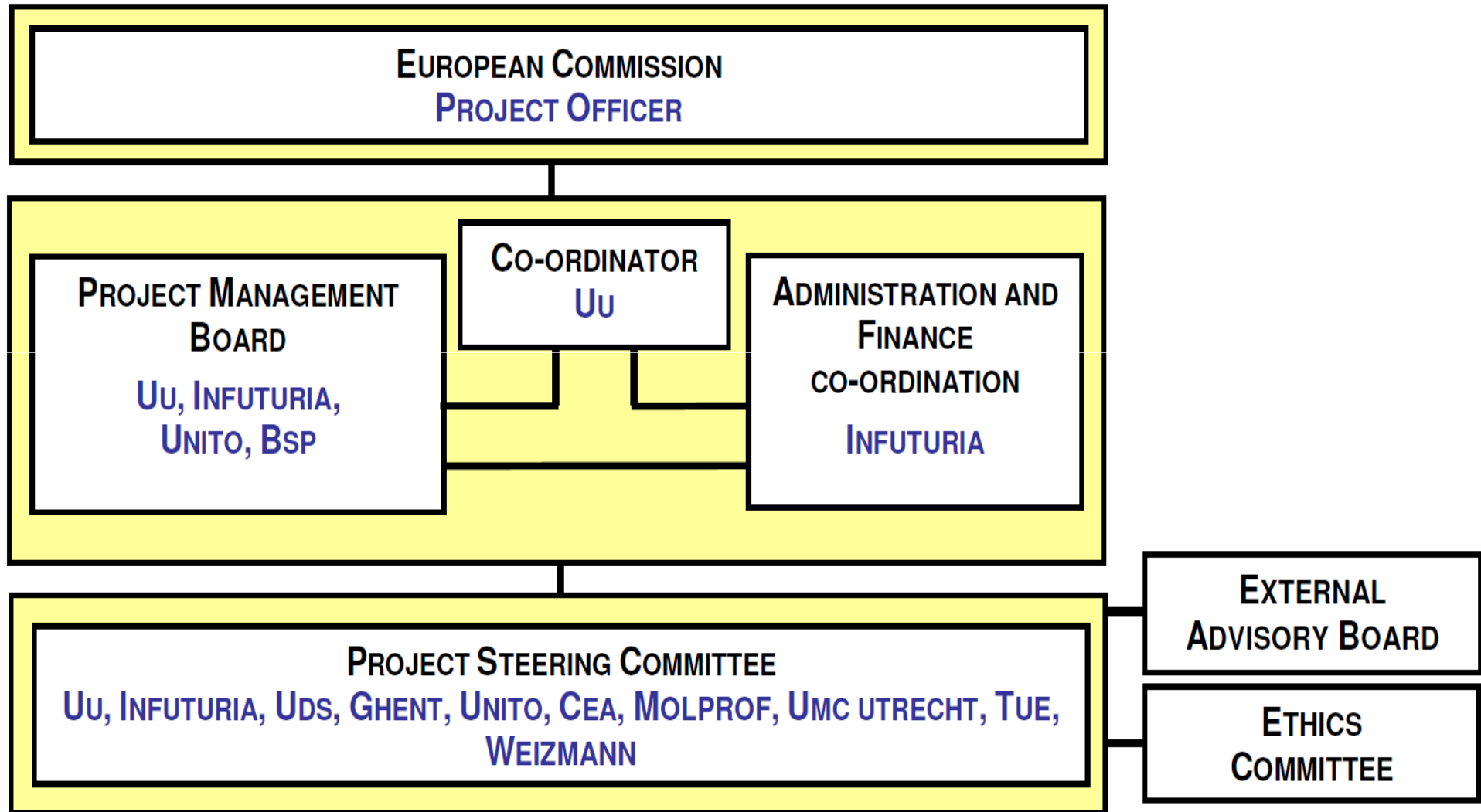
- Well-characterised targeted nanomedicines with broad applicability to disease treatment (rheumatoid arthritis, Crohn's disease, multiple sclerosis and cancer)
- Improved structural collaboration between industry and academia



Targeted Delivery of Nanomedicine



MEDITRANS Management Structure



Targeted Delivery of Nanomedicine



TARGETED DELIVERY OF NANOMEDICINE MEDITRANS

Broad applicability to chronic inflammatory diseases and cancer

**Rheumatoid
arthritis**

**Crohn's
disease**

**Multiple
sclerosis**

Cancer

Sub Project I: PLATFORM TECHNOLOGIES (Leader: UU)

WP1: (UU)
NANOCARRIER DESIGN

WP2: (UNITO)
DEVELOPMENT OF HIGH
SENSITIVITY IMAGING
PROBES FOR GUIDING
DRUG DELIVERY
PROCESSES

WP3: (MOLPROF)
FORMULATION OF DRUGS
AND IMAGING AGENTS INTO
CARRIERS /
PHYSICOCHEMICAL
CHARACTERISATION

Sub Project II: *IN VITRO* TARGETED DELIVERY (Leaders: UDS, GHENT)

WP4: (UDS)
RECOGNITION OF
TARGETS: CELLS,
TISSUES, ORGANS

WP5: (GHENT)
TARGET CELL UPTAKE
AND INTRACELLULAR
TRAFFICKING

WP6: (UMC UTRECHT)
STIMULUS INDUCED
RELEASE / ACTIVATION

Sub Project III: *IN VIVO* TARGETED DELIVERY (Leaders: UNITO, UU)

WP7: (TUE)
APPLICATION TO
RHEUMATOID ARTHRITIS
AND CROHN'S DISEASE

WP8: (UNITO)
APPLICATION TO MULTIPLE
SCLEROSIS

WP9: (WEIZMANN)
APPLICATION TO CANCER

Sub Project IV: TOXICITY RISK ASSESSMENT (Leader: CEA)

WP10: (CEA)
PRECLINICAL TOXICOLOGY
(in liaison with NANOSAFE 2, and
other EC projects)

Sub Project V: INDUSTRIAL EXPLOITATION (Leader: UU)

WP11: (UU)
INDUSTRIAL EXPLOITATION
(clinical grade materials, scale up
feasibility, long term stability, GMP,
clinical proof of concept)

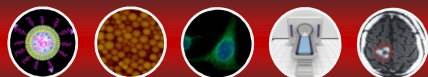
Sub Project VI: TRAINING AND DISSEMINATION (Leaders: UU, INFUTURIA)

WP12: (UDS)
TRAINING

WP13: (INFUTURIA)
DISSEMINATION

Sub Project VII: MANAGEMENT (Leaders: UU, INFUTURIA)

WP14: (INFUTURIA)
MANAGEMENT



Targeted Delivery of Nanomedicine



Work Package 1: Nanocarrier Design

Objectives

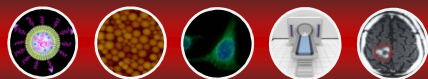
- To demonstrate the feasibility of the Emerging Materials (carbon-based nanoparticles) for targeted drug delivery
- To optimise the functionality of the Candidate Materials (polyplexes, nanogels, nanospheres, polymeric micelles, molecular imprinted particles, stimuli-sensitive liposomes) for targeted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

Work plan and conclusions from the third year

- WP1 focuses on *emerging materials*, like fullerenes & nanotubes, and on *candidate materials*, like polyplexes, nanogels, polymeric micelles, & stimuli-sensitive liposomes

Emerging materials:

- CEA has shortened commercially obtained carbon nanotubes to sizes well below 500 nm
- CEA established methods for functionalizing fullerenes and nanotubes with carboxyl, hydroxyl and amine groups, which can be used to modify the surface of these systems with hydrophilic polymers, like poly(ethylene glycol) (PEG)



Targeted Delivery of Nanomedicine



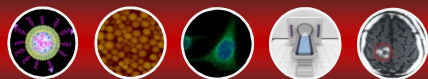
Work Package 1: Nanocarrier Design

Emerging materials continued...

- Several surface-functionalized and surface-modified materials have been sent to BRACCO for toxicity analysis
- At concentrations up to 1 mg/ml (*i.e.* the maximum concentration that could be reproducibly dispersed), hardly any cytotoxicity presented
- Both the LDH assay and the MTT assay were used, and experiments were performed in 6 different cell lines
- Future studies will focus on the ability of nanotubes to deliver DNA and/or siRNA into the cytoplasm of cancer cells

Candidate materials

- Significant progress has been made on Tasks relating to long-circulating polymeric micelles, surface-modified iron oxide nanoparticles, nanogels and polyplexes for siRNA delivery, and on stimuli-sensitive liposomes
- GHENT has been able to effectively PEGylate nanogels and to show that both PEGylated and unPEGylated nanogels are taken up by cancer cells (Figure 1A and 1B)



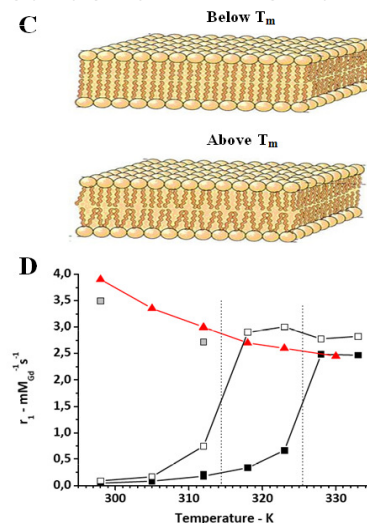
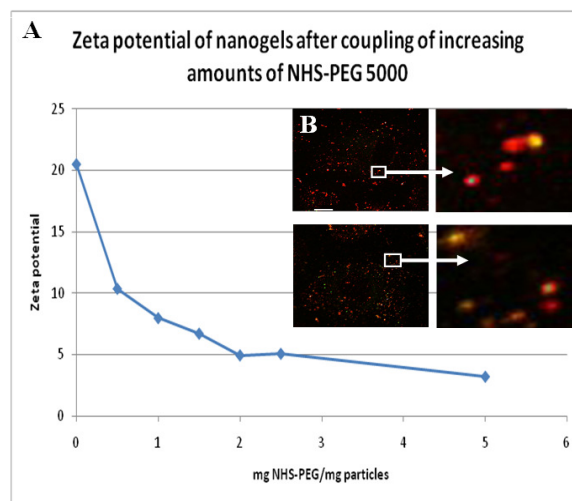
Targeted Delivery of Nanomedicine



Work Package 1: Nanocarrier Design

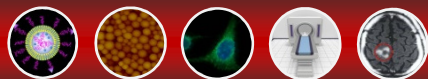
Candidate materials continued...

- UNITO has prepared systems which respond to increases in temperature, and which above the transition temperature (T_m) of the lipids, effectively release encapsulated imaging probes (Figure 1C and 1D)
- Future efforts within WP1 will focus both on these and on DNA- and siRNA-containing polyplexes, iron oxide-based nanoparticles and amino acid-based nanoparticle systems
- The most promising systems will be forwarded to other WPs for *in vivo* evaluation



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

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



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Targeted Delivery of Nanomedicine



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

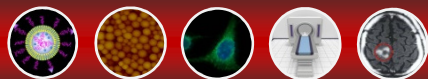
Objectives

- 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 third year

Task 2.1 Highly sensitive Gd(III)-based agents

- UNITO and BRACCO continued to pursue the synthesis of Gd(III) complexes endowed with high relaxivity
- A complex based on the coordination cage of DTPA was prepared (see Figure on next slide)

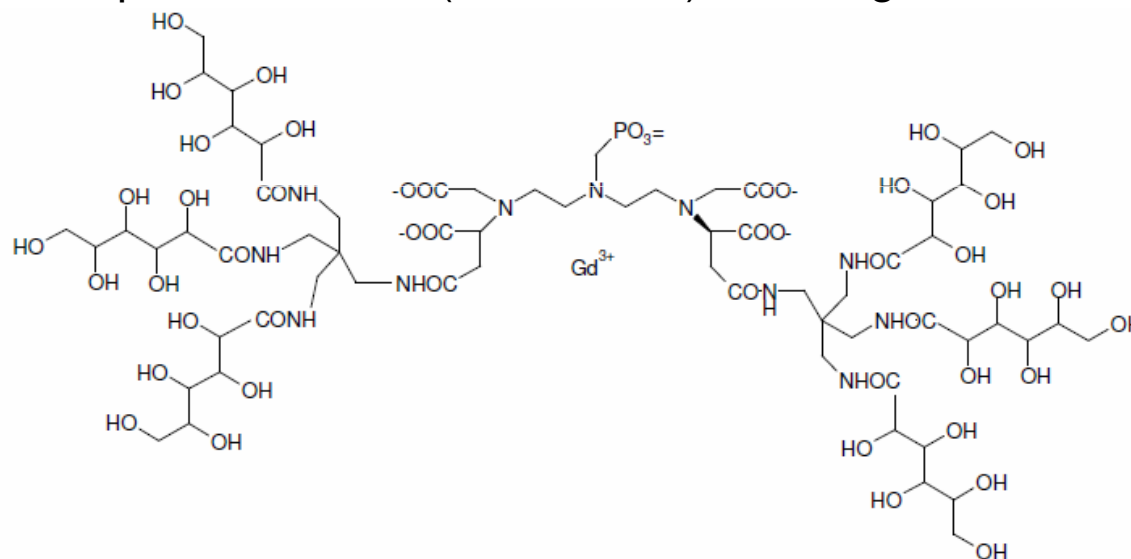


Targeted Delivery of Nanomedicine

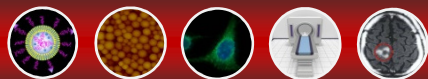


Work Package 2: Development of High Sensitivity Imaging Probes for Guiding Drug Delivery Processes

- The peculiarity of this chelate is the presence of poly-hydroxyl branches that, in addition to the central phosphonic moiety, ensure a high relaxivity ($19 \text{ mM}^{-1}\text{s}^{-1}$ at 20 MHz and 25 °C) in virtue of a significant relaxation contribution arising from exchangeable water protons bound (via H-bond) to the ligand



- UNED has been engaged in the physicochemical characterization of single-walled carbon nanotube SWNT's preparations
- Such systems displayed ferromagnetic behaviour and showed interesting MRI properties as contrast agents for diffusion weighted experiments



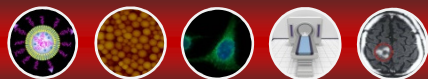
Targeted Delivery of Nanomedicine



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

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

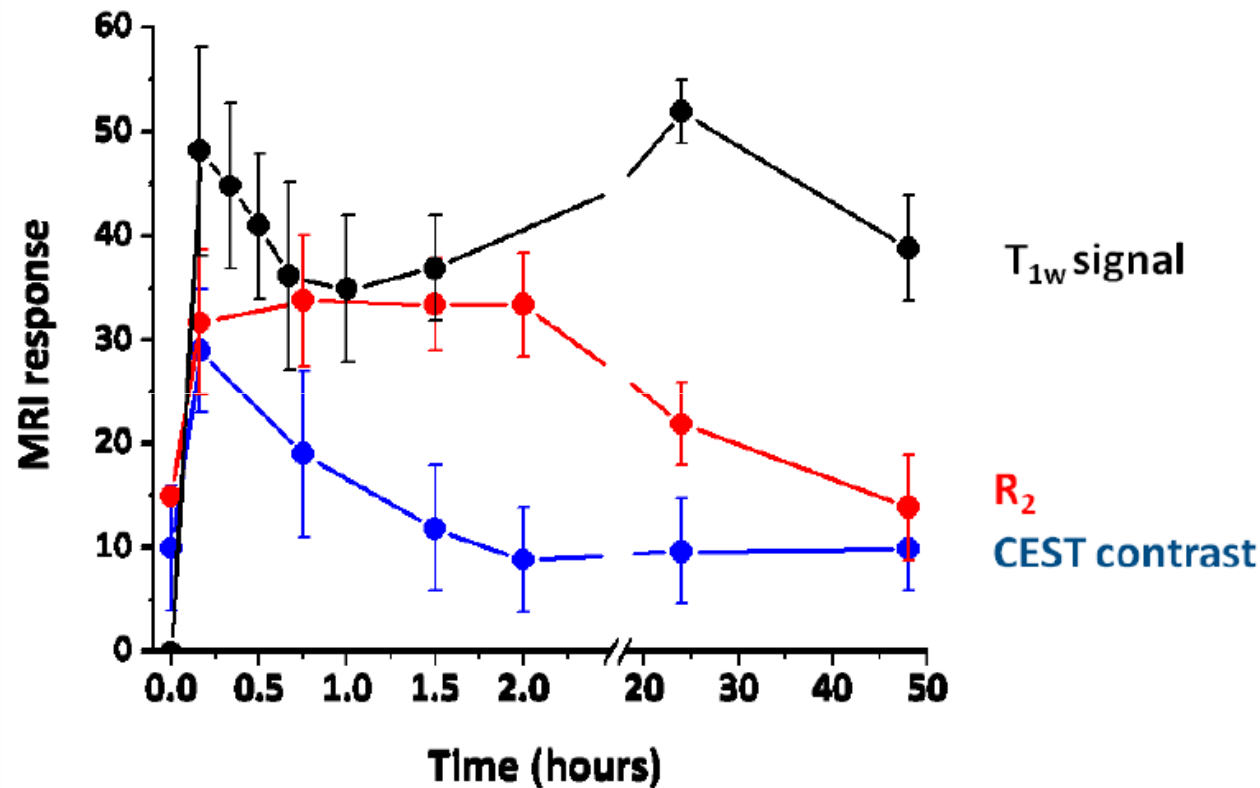
- PHILIPS and PHILIPSD carried out work aimed at developing temperature-sensitive liposomes for image-guided drug delivery
- A series of nanovesicles to monitor the release kinetics of encapsulated paramagnetic complexes from the aqueous lumen of the liposome as a function of the temperature were prepared
- These experiments showed that the CEST effect can be used to determine the melting phase transition temperature of the lipid membrane
- The research carried out in UNITO was mainly focused on paramagnetic liposomes suitably designed to act as multicontrast (T1, T2, and CEST) MRI agents
- The first experiments conducted *in vivo* on melanoma-bearing mice demonstrated the potential of such nanocarriers to provide an image contrast dependent on the release properties of the liposomes in the tumour environment



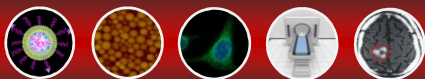
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Work Package 2: Development of High Sensitivity Imaging Probes for Guiding Drug Delivery Processes



Kinetic evolution of T_1 , T_2 (expressed by the R_2 value), and CEST contrast after intratumour injection of paramagnetic stealth liposomes



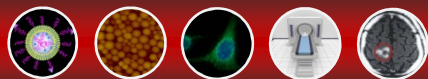
Targeted Delivery of Nanomedicine



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

Task 2.3 Novel iron-oxide based probes

- Carbon-coated iron oxide particles C@OxFe produced by laser pyrolysis at CEA have to be optimised as an imaging probe for biological applications
- C@OxFe must have a small size and exhibit colloidal stability
- Thus 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
- The dispersion exhibited a strong positive magnetic signal but was only stable for hours
- Optimisation of the iron oxide particles stability in a biological medium is in progress



Targeted Delivery of Nanomedicine



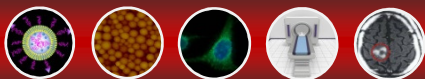
Work Package 2: Development of High Sensitivity Imaging Probes for Guiding Drug Delivery Processes

Task 2.4 Optical Imaging Probes

- UNITO continued the work aimed at investigating the kinetics of the intracellular trafficking of liposomes
- To this purpose liposomes loaded with pH sensitive fluorescent dyes were prepared
- The ultimate goal of this study is to get information about the intracellular localization of the vesicle (and its integrity) by measuring the pH of the environment in which the liposome (or its payload) is localized over time
- Fluorescence confocal microscopy experiments on living cells (time lapse modality) are in progress in order to assess the kinetics of the release of the fluorescent dye from the nanovesicles

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

WP2 Participants: CEA, MAGFORCE, TUE, PHILIPS, BRACCO, UNITO, UNED, GUERBET, PHILIPSD



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Targeted Delivery of Nanomedicine



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

Objectives

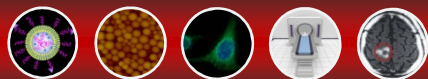
- To load the nanocarriers with biologically active compounds and imaging agents
- To study the physicochemical characteristics of the nanomedicines which are relevant for the overall aims of MEDITRANS

Work plan and conclusions from the third year

- WP3 encompasses the formulation of nanocarriers, based on established, candidate and emerging systems, loaded with drugs and imaging agents, and their subsequent characterisation
- Formulation of nanocarriers with drugs, will focus on loading with drugs for the treatment of Crohn's Disease, Cancer and MS

Established Nanocarrier Systems

- Significant progress has been made on the formulation and characterisation of established nanocarrier systems
- Long-circulating liposomes for passive targeting loaded with corticosteroids have demonstrated favourable size and sufficient encapsulation, and are currently being investigated for *in vitro* and *in vivo* response

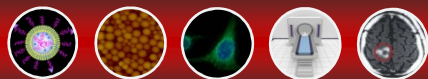


Targeted Delivery of Nanomedicine



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

- Long-circulating liposomes for active targeting have been formulated with an antibody to target EGFR-overexpressing ovarian carcinoma cells
 - These have been loaded with Boron (NCT) and have already been shown to be effective in *in vitro* studies
 - More detailed physicochemical characterisation is underway
 - A range of analytical techniques are being employed to understand structure, function, size, and stability
-
- Imaging probes, such as amphiphilic paramagnetic Gd(III) complexes, have been successfully incorporated into the bilayer of long-circulating liposomes
 - These systems are currently under investigation in subsequent work packages (e.g. WP9)
 - In addition, a Gd(III) complex bearing a cholesterol-like moiety has also been successfully incorporated in a liposome formulation
 - The aim was to incorporate in a liposome an imaging probe that could mimic the chemical structure of steroid-like drugs that are often delivered by liposomal systems



Targeted Delivery of Nanomedicine

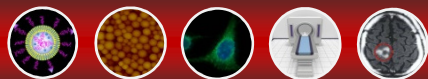


Work Package 3: Formulation of Drugs and Imaging Agents into Carriers / Physicochemical Characterisation

- Proteoliposomes demonstrating channel activity in response to a model chemical activator (MTSET) have been developed
- Channel protein liposomes are being formulated to contain corticosteroids
- PLGA nanoparticles for the treatment of Crohn's disease have been formulated
- Although the drug loaded PLGA nanoparticles show favourable size (sub-200 nm), encapsulation efficiencies of greater than 70% and stability during storage, unfavourable drug release profiles were observed
- Work with PLGA nanoparticles has changed focus, and formulations encapsulating corticosteroids are being developed

Candidate Nanocarrier Systems

- A range of nanoparticles for intravenous siRNA delivery have been developed
- PLGA nanoparticles have also been successfully formulated to contain siRNA
- These formulations have demonstrated sustained release of siRNA, and that the siRNA can remain protected by the PLGA from degradation for up to 72 hours
- In addition, the PLGA nanoparticles have been surface modified with excipients that enhance the gene silencing properties of the formulation

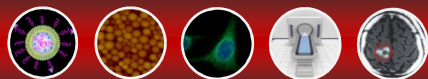


Targeted Delivery of Nanomedicine



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

- Folate receptor-targeted delivery of siRNA and pDNA has also been explored
- PLGA nanoparticles have been successfully complexed with folate-PEG-PEI polyplexes and pDNA
- Biodegradable amine modified branched polyesters (based on DEAPA-68-10) have been formulated with siRNA
- The nanocarriers have been developed with a particle size of 150-225 nm, zeta potential of 40-50mV, and are sufficiently stable for endocytotic uptake
- During *in vitro* studies the formulation has demonstrated efficient knockdown of the Luciferase reporter gene, with minor, or no, cytotoxicity
- Dextran microgels and nanogels have been formulated with siRNA
- Homogenous loading of the siRNA has been achieved in microgels
- Nanogels formulated with a particle size of 200 nm and a zeta potential of 0-30mV
- High loading of siRNA can be achieved without inducing aggregation
- In order to use dextran for siRNA delivery *in vivo*, it is expected that these carriers will need to be PEG-coated to prevent opsonisation and fast clearance from the blood
- A PEG coating has been successfully coupled to the dextran nanoparticles without impacting on the siRNA loading or release



Targeted Delivery of Nanomedicine



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

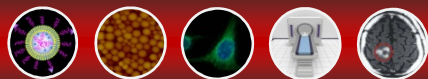
- Nanoparticles for intravenous pDNA delivery, has seen the development of protocols for the formulation of cationic polymer/pDNA complex dispersions
- Imaging probes containing nucleic acid have been explored
- Polymeric micelles have been formulated to contain 10 nm oleic coated iron oxide particles, and are currently in the process of being loaded with drugs
- Iron-oxide particles, for the treatment of cancer, have been developed coupled with stealth coatings of PEG and aminosilanes
- Unfortunately technical challenges were encountered in the development of dextran nanoparticles with a molecular imprint, therefore this Task has ceased

Emerging Nanocarrier Systems

- Nanotubes and fullerenes are being loaded (by covalent coupling) with corticosteroids (prednisolone, dexamethasone), possibly other chemotherapeutic agents, and Gd-chelates
- Characterisation will include size, amount of loading, and surface morphology

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

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



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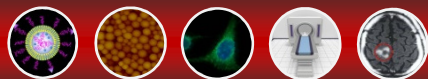
Targeted Delivery of Nanomedicine



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

Work plan and conclusions from the third year

Task 4.1 Recognition of targets in cancer

- Ovarian cancer cell lines (MLS, SKOV-3) were successfully transfected for EGFP, luciferase, YFP, EGFP/DsRed and Luciferase/DsRed by WEIZMANN. The efficacy of siRNA in suppressing expression of reporter genes EGFP and Luciferase was determined by fluorescence and bioluminescence imaging
- MAGFORCE NanoTherm® PEG coated nanoparticles were incubated with C3HRS1 mouse mammary carcinoma cells by CHARITE. PEG-coating showed a negative effect in terms of cellular uptake with this cell line (Figure 1)

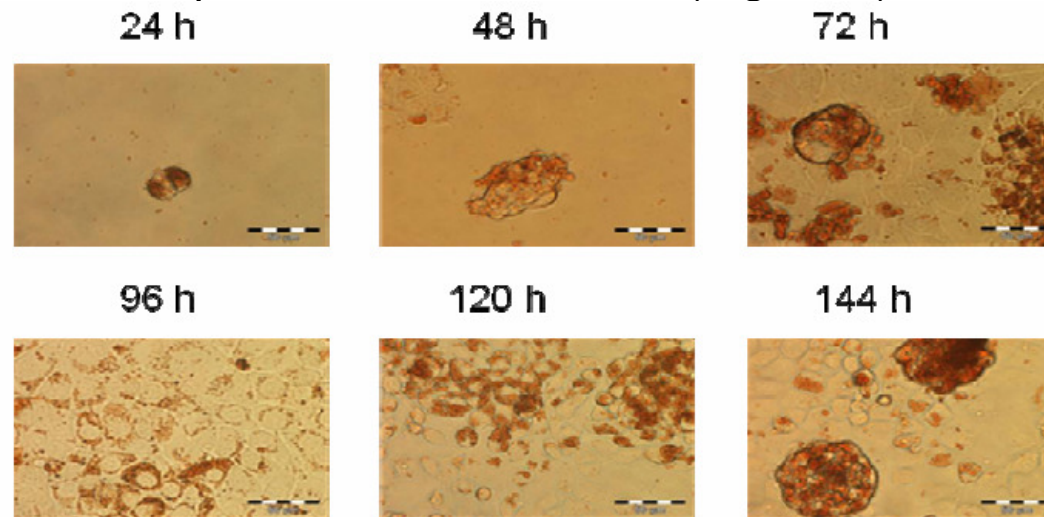
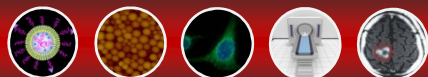


Figure 1: Uptake of low level PEGylated iron oxide nanoparticles into C3HRS1 mammary carcinoma cells (CHARITE)



Targeted Delivery of Nanomedicine



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

Task 4.2 Recognition of targets in CD

- A three dimensional co-culture model of intestinal epithelial cells, macrophages and dendritic cells has been developed to simulate the inflamed intestinal mucosa (Figure 2, UDS). Inflammation is induced by stimulation of the co-culture with IL-1 β and lipopolysaccharides of *Salmonella typhimurium*
- The model was characterized with regards to co-culture transepithelial electrical resistance (TEER), release of inflammatory marker protein IL-8 and transport properties

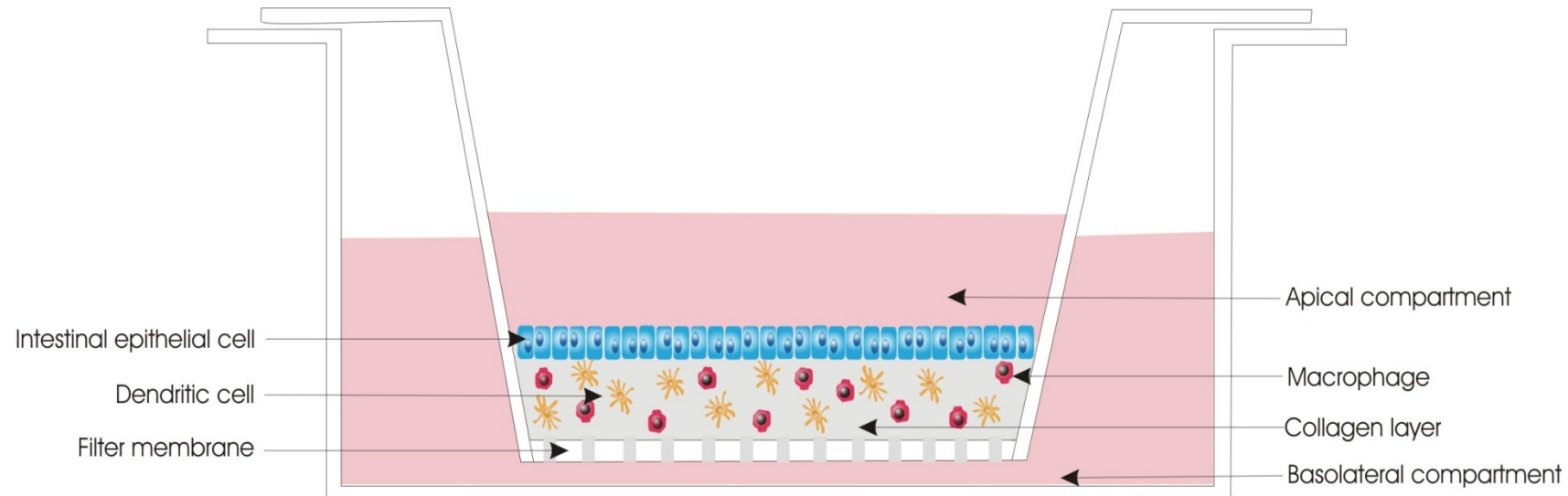
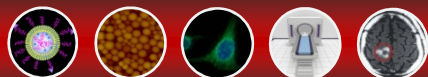


Figure 2: Schematic overview of the novel 3D co-culture of the inflamed intestinal barrier (UDS)



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

Task 4.2 Recognition of targets in CD continued...

- In the non-inflamed state, TEER values for the co-culture were comparable to Caco-2 monolayers. Upon inflammation, a 20-25% drop in TEER values was observed accompanied by changes in tight junction organization comparable to *in vivo* inflamed tissue. This resulted in increased transport rates across the inflamed barrier
- Confocal Laser Scanning Microscopy images of the co-culture revealed viability and active mobility of the immunocompetent cells, which were able to reach the apical side of the co-culture (Figure 3)

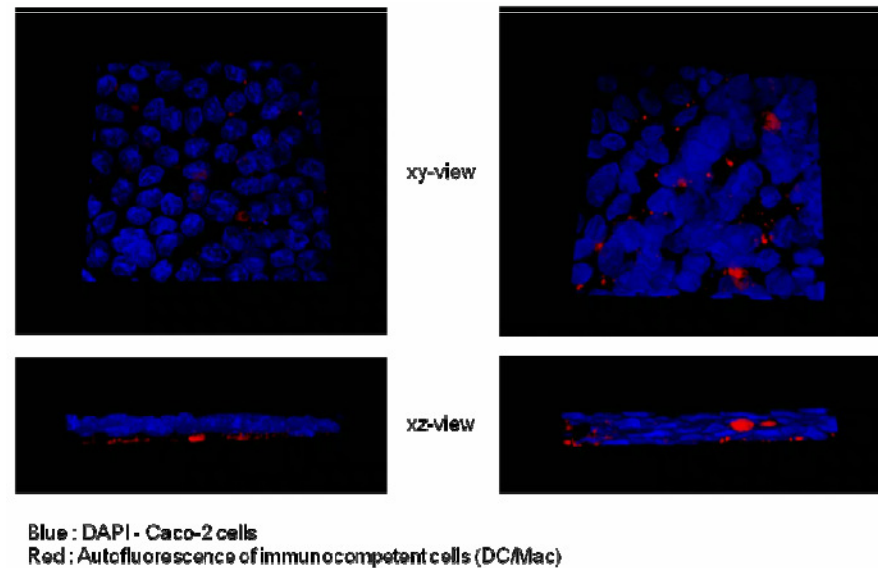
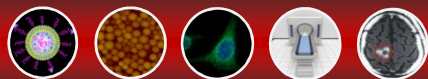


Figure 3: CLSM-images of 3D co-culture of the inflamed intestinal barrier (UDS)



Targeted Delivery of Nanomedicine



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

Task 4.3 Recognition of targets in RA

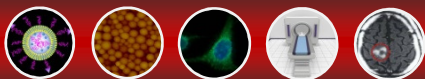
- The uptake of different stealth liposomal formulations, prepared at UU, into macrophages has been evaluated using Fluorescence Activated Cell Sorting (FACS)
- Surface Plasmon Resonance measurements at UU revealed the interactions of PEG2000- and PEG5000-Liposomes with the following plasma proteins: HSA, ApoE, β 2-Glycoprotein, Fibronectin and α 2-macroglobuline
- *In vivo* circulation half lives have been studied at UU and are to be correlated with *in vitro* data to identify the best predictor of extravasation ability

Task 4.4 Recognition of targets in MS

- An *in vitro* model of the inflamed brain barrier has been set up at ACROSS based on porcine brain endothelial cells (PBECE) and induction of inflammation with pro-inflammatory cytokines
- TEER and transport properties of the model were evaluated
- The high variability found in TEER-values and P-gp efflux ratios is to be reduced by co-culture of PBECE with glial cells

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

WP4 Participants: UU, MAGFORCE, CHARITE, CSEM, UDS, BSP, ACROSS, RBM, WEIZMANN



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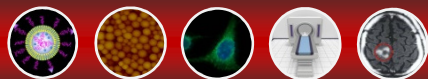
Work Package 5: Target Cell Uptake and Intracellular Trafficking

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 and conclusions from the third year

- Year 1: The loading of siRNA in dextran based microgels was demonstrated
- Year 1: Gene silencing activity of these microgels with siRNA targeted against green fluorescent protein (GFP) was shown
- Year 2: Proven that siRNA can also be loaded in dextran based nanogels (typically 200 nm)
- Year 2: These nanogels are efficiently taken up by cells, resulting in a biological activity that can be prolonged over time, and which can be enhanced by the use of photochemical internalisation (PCI)



Work Package 5: Target Cell Uptake and Intracellular Trafficking

- Year 3: PEGylated nanogels were evaluated because PEGylation has beneficial effects for further *in vivo* use, such as, preventing rapid blood clearance
- Cellular uptake was measured in A431 and Huh_7 cells
- Figure 1 A shows that 80% of the cells efficiently take up the nanogels. The mean intensity per cell, however, is lower in the case of PEGylated versus non-PEGylated nanogels and for Huh_7 cells versus A431 cells, indicating that fewer nanogels are present per cell

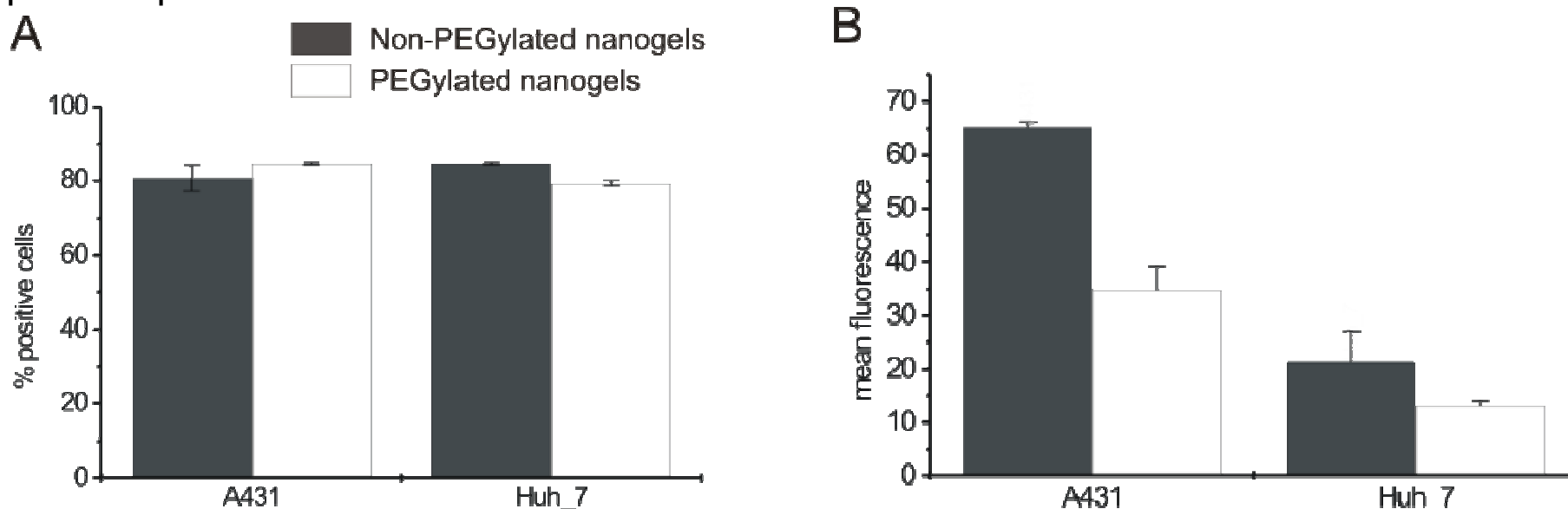
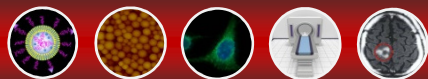


Figure 1: Flow cytometry results obtained after treating HuH-7 cells with AF488-siRNA loaded PEGylated and non-PEGylated nanogels. (A) depicts the percentage of positive cells in the total population. (B) shows the mean fluorescence per cell. All samples were prepared in triplicate and incubated with trypan blue before flow cytometry measurements



Targeted Delivery of Nanomedicine



Work Package 5: Target Cell Uptake and Intracellular Trafficking

- Figure 2 shows that the gene silencing capacities of PEGylated nanogels are comparable with that of non-PEGylated nanogels and increase with nanogel concentration
- The effect with the higher concentrations of nanogels is comparable with that of the commercially available RNAiMAX, indicating that a substantial level of downregulation is reached
- The effect of PEGylated nanogels is being studied in the blood with the aim of increasing the silencing effect in specific tissues by adding targeting moieties to the PEGylated nanogels

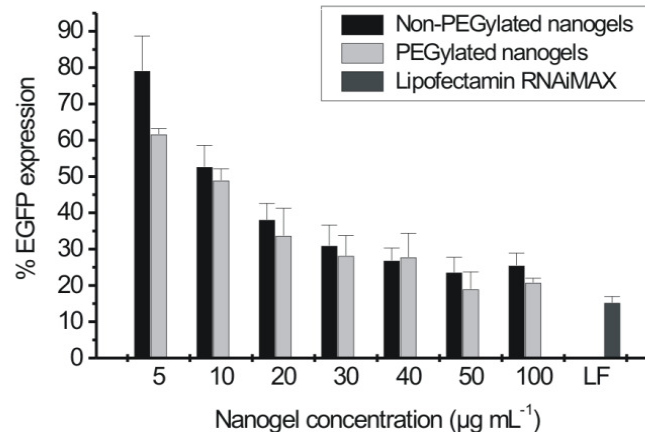
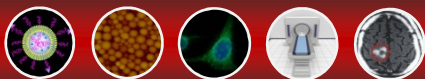


Figure 2: EGFP gene silencing by both non-PEGylated and PEGylated nanogels in HuH-7_EGFP cells. Increasing concentrations of nanogels were applied (4h incubation) and Lipofectamin RNAiMAX was used as a positive control. The used amount of siRNA per well was 50 pmol and the EGFP expression of cells treated with nanogels loaded with mock NC-1 siRNA was set as 100%

WP5 Leader: GHENT - Prof. Dr Stefaan de Smedt (stefaan.desmedt@ugent.be)

WP5 Participants: UU, PCI, GHENT, MARBURG, UNITO



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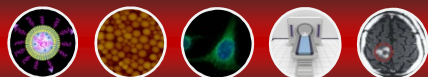
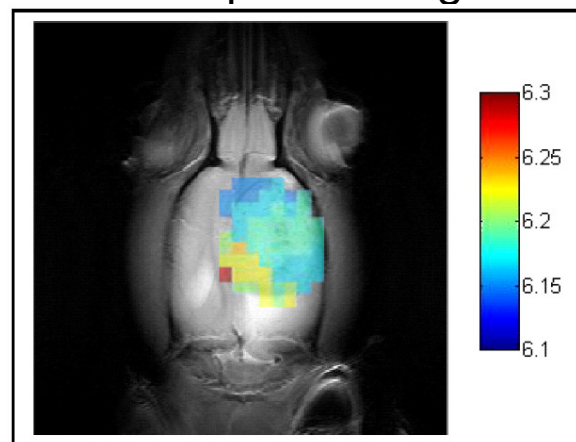
Work Package 6: Stimulus Induced Release / Activation

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 and conclusions from the third year

- CSIC set up a model of experimental glioma in the brains of rats and a protocol to measure *in vivo* the extracellular pH in these tumours (see Figure below). This will allow monitoring of the *in vivo* effect of pH on drug release from pH sensitive carriers

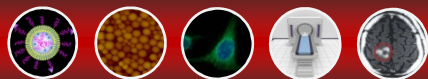
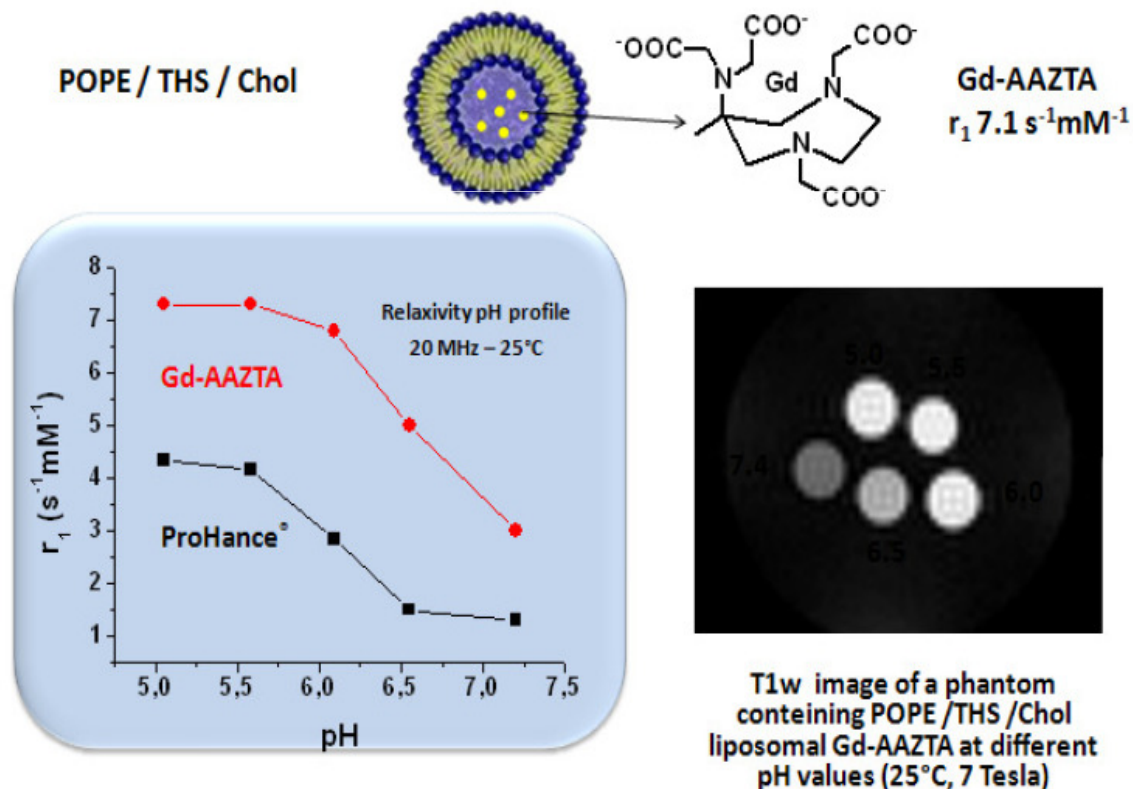


Targeted Delivery of Nanomedicine



Work Package 6: Stimulus Induced Release / Activation

- UNITO developed and optimized the release property of nanomedicines loaded with MRI probes. Endogenous factors (e.g. pH, specific enzymes) triggered release
- The Figure below shows the *in vitro* release performance of a pH sensitive liposome encapsulating a paramagnetic Gd-complex (Gd-AAZTA) endowed with a good ability to generate a T_1 contrast

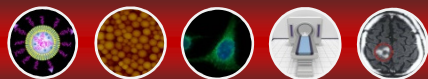
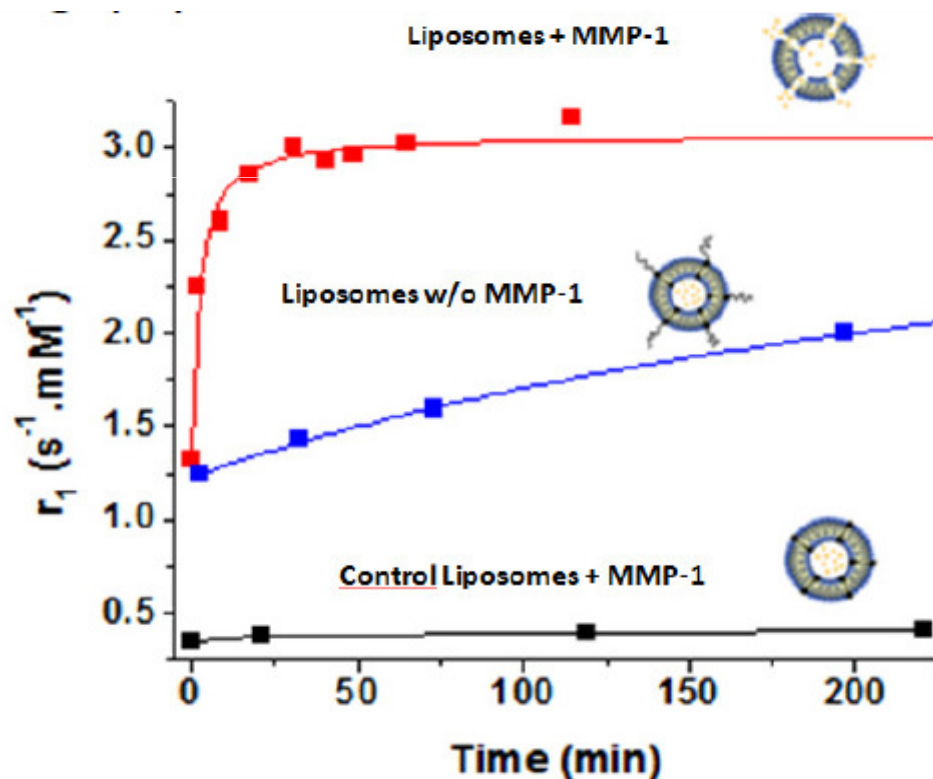


Targeted Delivery of Nanomedicine



Work Package 6: Stimulus Induced Release / Activation

- A second system was designed to trigger release upon the action of a specific enzyme
- An amphiphilic compound made of a stearic acid linked to an octapeptide was synthesized by solid phase synthesis and incorporated in non-stealth liposomes
- Release of an imaging MR probe, in the presence of an enzyme (MMP-1) able to hydrolyze the oligopeptide, has been demonstrated *in vitro*

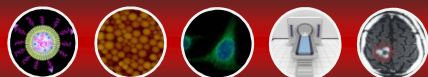
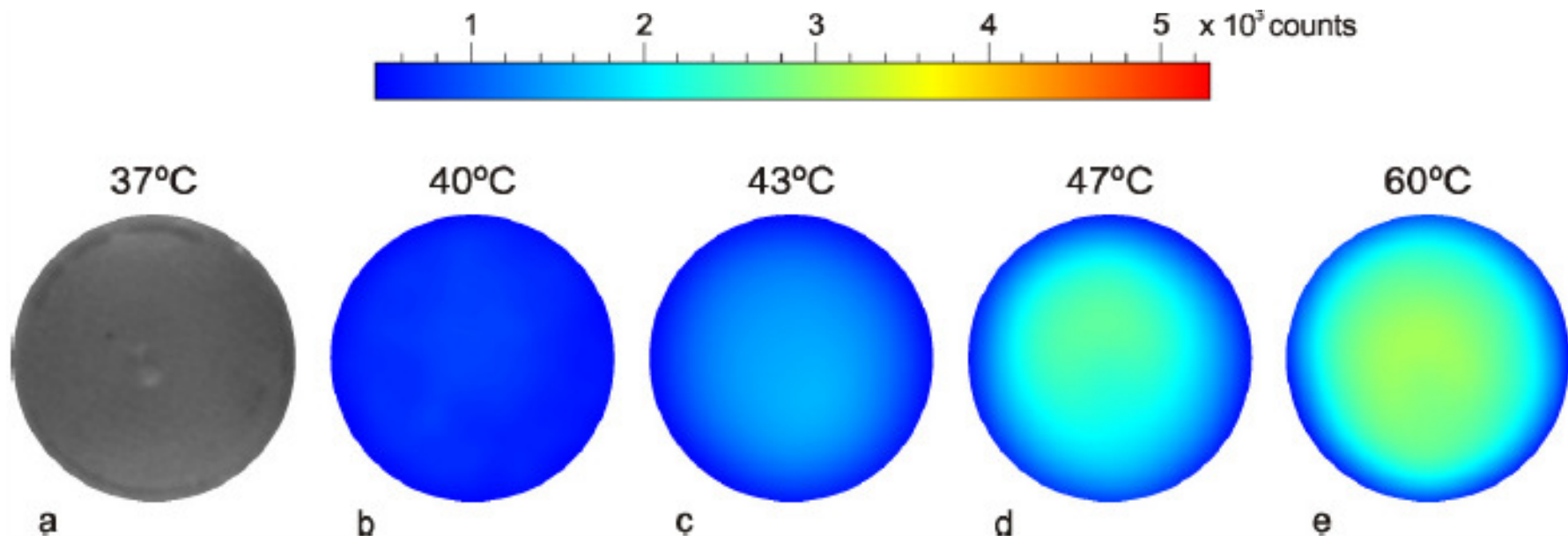


Targeted Delivery of Nanomedicine



Work Package 6: Stimulus Induced Release / Activation

- UMC UTRECHT developed thermosensitive liposomes, which release their content after heat triggering (for example by high intensity focused ultrasound)
- The Figure below shows release of a fluorescent dye from liposomes after heat triggering for 30 seconds at different temperatures
- At body temperature, no release is observed
- Increasing fluorescence is observed after higher temperatures are applied

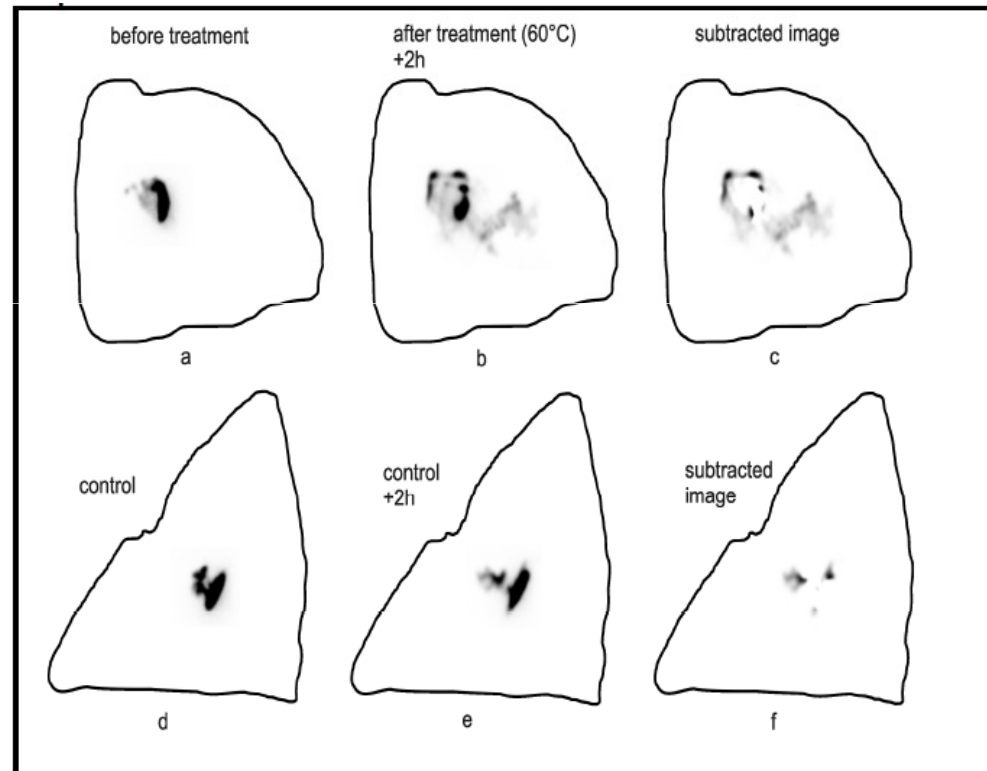


Targeted Delivery of Nanomedicine



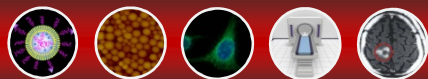
Work Package 6: Stimulus Induced Release / Activation

- In the Figure below an *ex vivo* porcine hind limb is injected with ^{99m}Tc containing liposomes
- Distribution of ^{99m}Tc throughout the tissue is observed after exposure to 60°C



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



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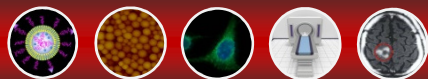
Work Package 7: Application to Rheumatoid Arthritis and Crohn's Disease

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

Work plan and conclusions from the third year

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



Targeted Delivery of Nanomedicine



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

- MRI gives information about the soft tissues and fluid present in joints
- CT 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)

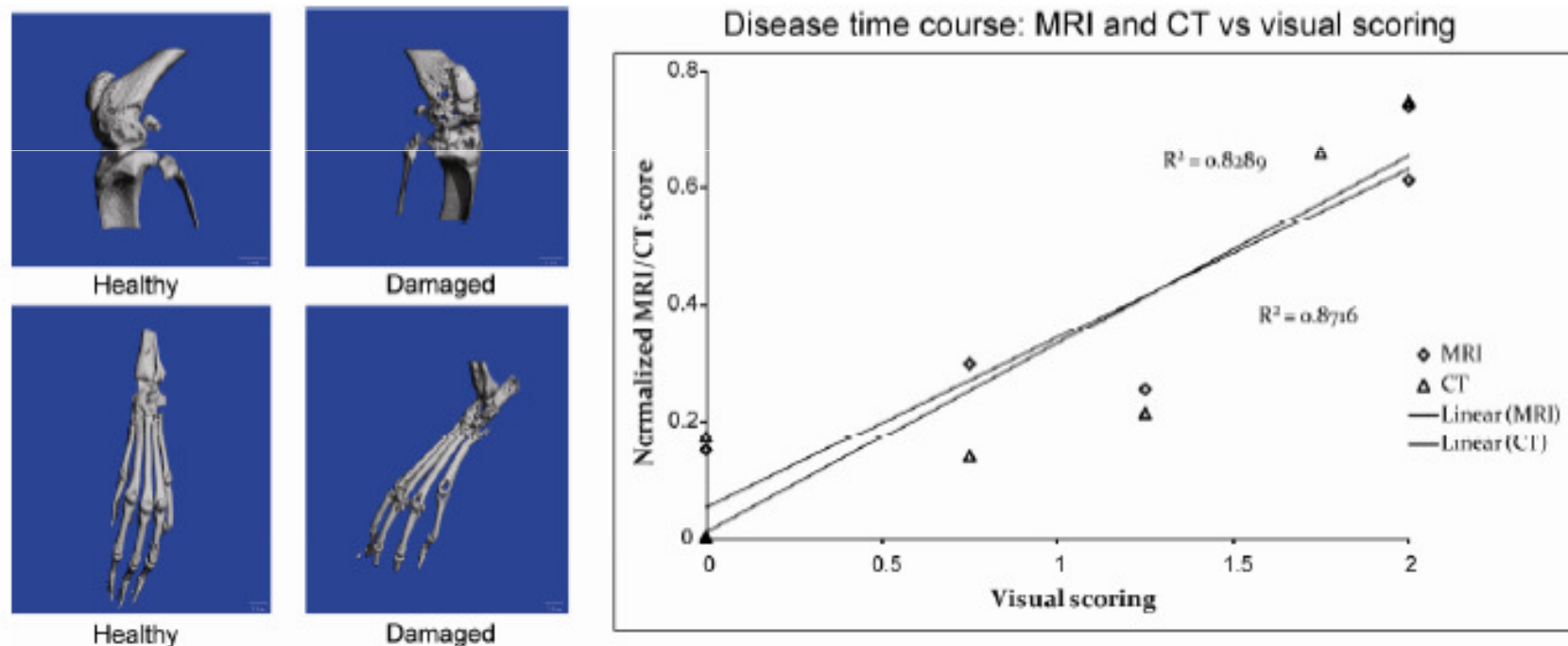
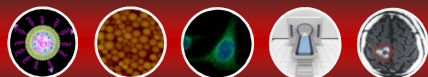


Figure 1: Left panel shows micro CT images of the paws and knees of mice, depicting a healthy (left) and a diseased animal (right). Right panel shows paw inflammation clinical scores vs total area of the MRI and CT images of the paws of mice



Targeted Delivery of Nanomedicine



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

- As preclinical monitoring of disease progress was successful, an anti-inflammatory therapy was evaluated with MRI
- A single dose of Dexamethasone did not result in a large decrease of paw inflammation scores (Figure 2, lower panel). However, the same single dose, encapsulated in PEG-liposomes, resulted in a complete disappearance of paw inflammation at day 5 post-treatment, thereafter inflammation increased again

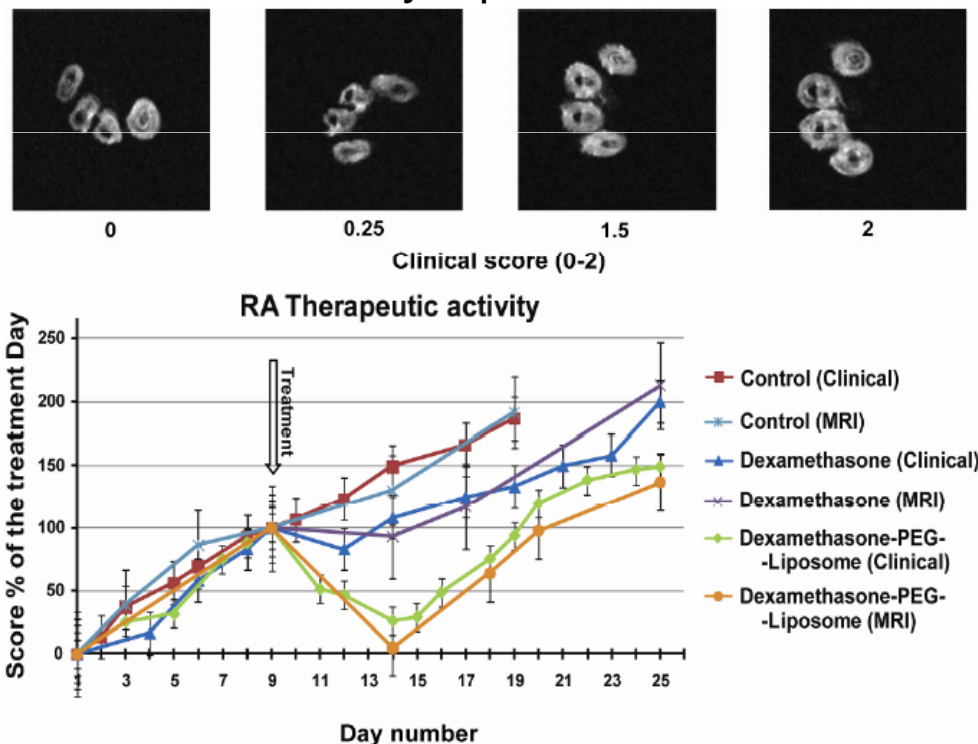
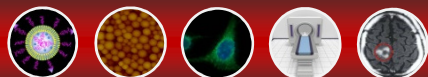


Figure 2: upper panel shows sagittal T₁ weighted MRI images of the paws of mice, taken at the same position, depicting the progression of the disease with higher clinical score. Lower panel shows paw inflammation clinical scores and total area of the MRI images of the paws of mice after single treatment. The score was set at 100 % at the day of treatment. Arrow indicates the day of treatment

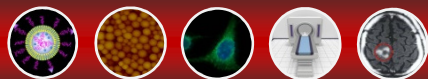


Targeted Delivery of Nanomedicine



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

- This study demonstrates the profound anti-inflammatory activity of Dexamethasone-PEG-Liposomes, where the reduction of paw inflammation was rapid and the therapeutic effect lasted more than a week
- Encapsulation of the drug in the nanocarrier system can strongly enhance its beneficial effect in RA
- Further work has been performed to establish appropriate read outs of *in vitro* models for measuring TNF- α silencing
- The murine macrophage cell line RAW264.7 was used and TNF- α production was induced with LPS
- Extracellular measurements of TNF- α by ELISA were insufficient for measuring gene silencing. Therefore, protocols have been established for intracellular TNF- α staining by flow cytometry
- Ongoing work focuses on toxicity assays and cytometric bead assays to measure extracellular cytokines induced by the different nanocarrier systems
- Primers have been designed for Q-PCR determination of TNF- α silencing at the mRNA level
- Comparative studies of MEDITRANS carriers (dendrimers, PLGA nanoparticles, polymers from UU and nanogels from GHENT) will be performed in RAW264.7 cells
- The *in vivo* siRNA studies using an RA model are planned at TUE



Targeted Delivery of Nanomedicine

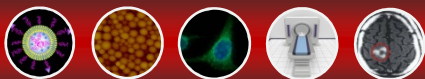


Work Package 7: Application to Rheumatoid Arthritis and Crohn's Disease

- CSIC developed the 3D MRI visualization of the progression of Gd (III) doped materials through the gastrointestinal tract in live mice
- However, CD studies suffered from the lack of a proper animal model
- UU now have a new animal model, which will be tested at UU, TUE and CSIC

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

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



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Work Package 8: Application to Multiple Sclerosis

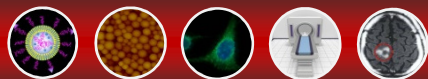
Objectives

- 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

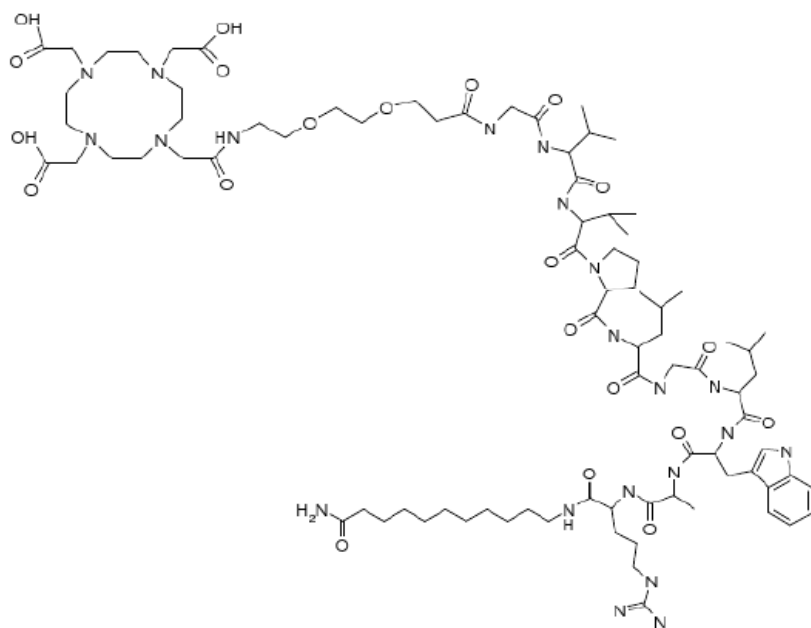
Work plan and conclusions from the third year

Imaging guided drug delivery

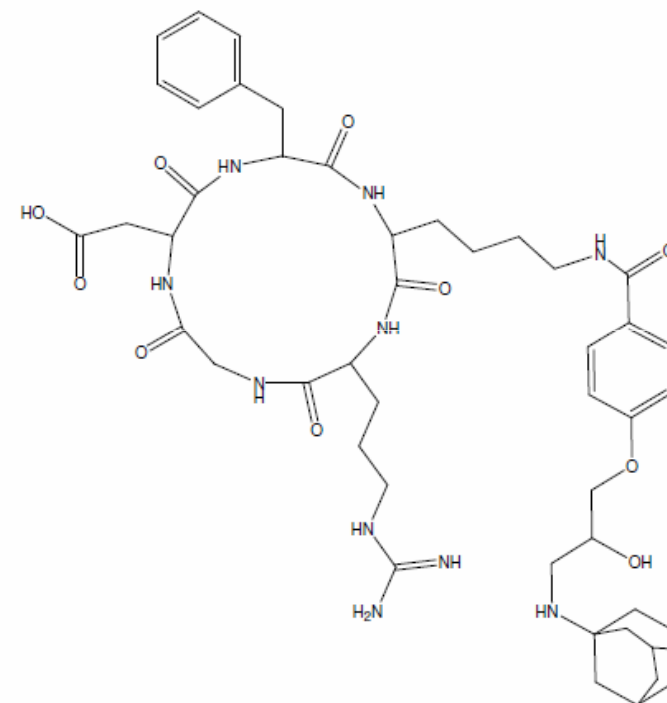
- A successful protocol for the *in vivo* MRI visualization of the delivery of drugs against MS requires the design of a carrier that couples an efficient and selective target to the MS lesion with the ability to accumulate a high number of imaging probes
- Poly-b-cyclodextrins (poly-b-CDs) are a useful carrier to achieve this task
- The main advantages of these systems are:
 - i) good biocompatibility
 - ii) the ability to promote a non covalent binding of different systems on the same carrier molecule



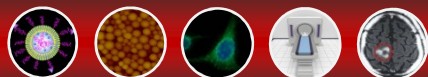
Work Package 8: Application to Multiple Sclerosis



Gd-K11n for the MRI visualization of MMPs



cRGDfK-Ada for targeting

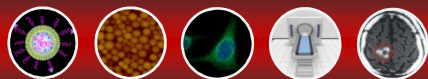


Targeted Delivery of Nanomedicine



Work Package 8: Application to Multiple Sclerosis

- Therefore, research focused on the synthesis of ligands that are able to:
 - i) strongly bind poly-b-CDs
 - ii) exhibit a selective targeting ability towards MS lesions
 - iii) generate an efficient MRI contrast at the pathological site that is responsive to the activity of specific MMPs
- To image drug delivery in MS lesions, a multicomponent system has been conceived
- This system is composed of a CA responsive to the activity of MMPs, an inhibitor of MMPs, and a vector targeted to inflammation regions in the CNS:
 - i) Compound Gd-K11n has been synthesized and selected as the pro-CA for the visualisation of MMP activity
 - ii) Compound cRGDfK-Ada targets to integrins
 - iii) MMP Inhibitor II/III/V (Merck-Calbiochem)
- All these compounds interact with poly-b-CDs, that constitute the platform hosting all components until they reach the targeted region
- In the next step of this WP, a MRI method for the *in vivo* evaluation of the MMP inhibitor delivery to the inflammation areas of MS brains will be evaluated



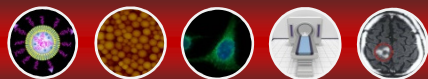
Targeted Delivery of Nanomedicine



Work Package 8: Application to Multiple Sclerosis

Passive targeting of corticosteroids

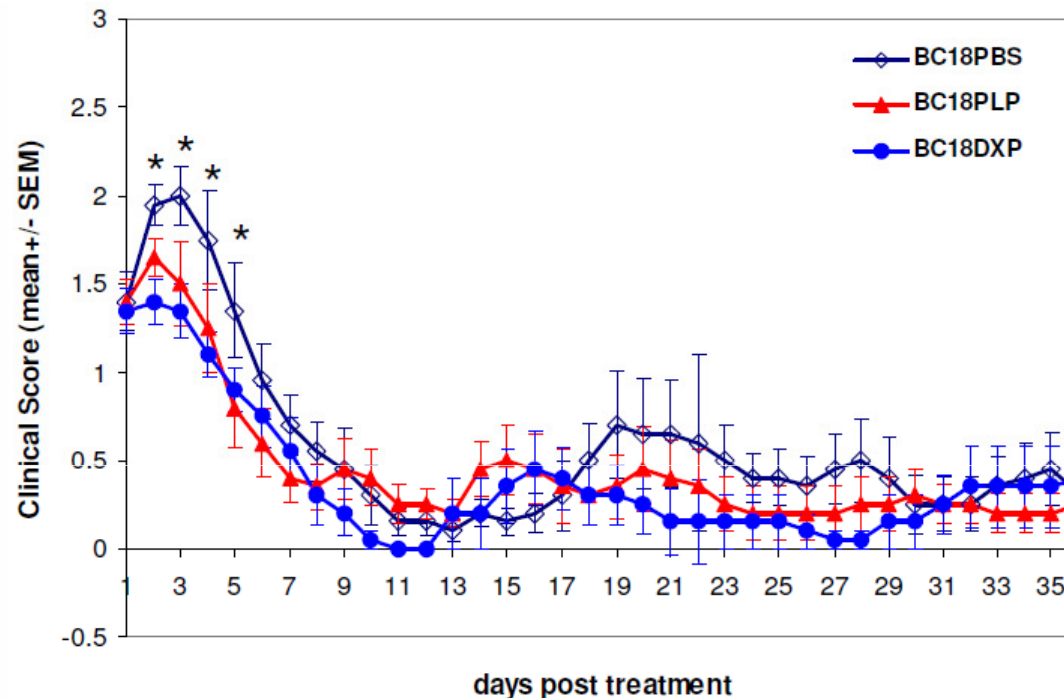
- Corticosteroids are often used to improve the rate of recovery from acute exacerbation in MS patients
- Liposomal encapsulation leads to enhanced efficacy of glucocorticosteroids (GS) in treatment of autoimmune diseases
- The effects of long-circulating liposomal prednisolone were evaluated in a relapsing-remitting mouse model (SJL-EAE mice), a model closely reflecting aspects of MS
- At the first peak of the disease (clinical score ~ 1-2), significant clinical differences were observed in animals receiving a single injection of liposomal prednisolone phosphate (BC18PLP) or liposomal dexamethasone phosphate (BC18DXP) compared to vehicle-treated mice (BC18PBS)
- These differences were maintained for four consecutive days during the acute phase of the disease
- No relevant differences were found between animals treated with BC18PLP/BC18DXP or BC18BPS at a later time
- No significant clinical differences were found with the single injection of free corticosteroids compared to vehicle treated mice



Targeted Delivery of Nanomedicine



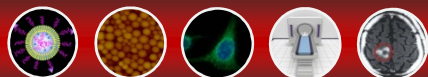
Work Package 8: Application to Multiple Sclerosis



Time course of the clinical score in SJL-EAE mice treated with liposomes loaded with PBS or prednisolone (PLP) or dexamethasone (DXP)

- The clinical benefits are more pronounced with the liposomal drugs as compared to free corticosteroids, at least up to 4 days after the onset of the pathology

WP8 Leader: UNITO - Dr Giuseppe Digilio (giuseppe.digilio@unito.it) **Previous WP8 Leader: RBM** - Dr Beatrice Greco (beatrice.greco@merckserono.net)
WP8 Participants: UU, FOM, PHILIPS, RBM, BRACCO, UNITO, GUERBET, PHILIPSD, IDT, UMC UTRECHT, MSSA



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Work Package 9: Application to Cancer

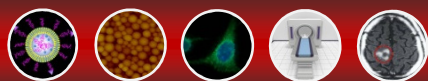
Objectives

- 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 and conclusions from the third year

Pharmacokinetics and pharmacodynamics of liposomal encapsulated drug formulations (UU, BSP)

- Concentration time courses of encapsulated drug and released drug have to be measured in plasma, tumour and healthy tissue showing liposomal uptake
- A method, based on the enzymatic hydrolysis of only released prednisolone phosphate, was successfully developed to measure the encapsulated and free drug concentrations of liposomes in PBS
- This method was extrapolated for the determination of encapsulated and free drug concentrations in plasma, tumour and healthy tissue using protein precipitation and LCMS
- An *in vivo* experiment using tumour bearing mice provided samples of plasma, tumour, liver, spleen, kidney, heart, lungs, muscle, bone marrow and intestine



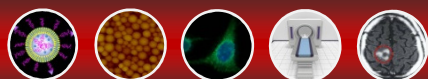
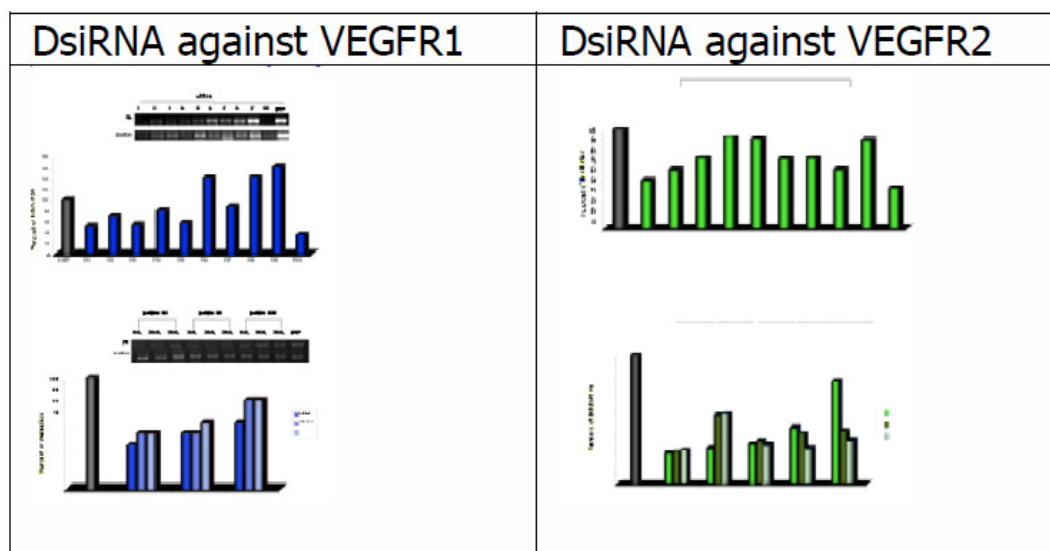
Targeted Delivery of Nanomedicine



Work Package 9: Application to Cancer

Synthesis and analysis of siRNA (IDT, UU, WEIZMANN)

- IDT provided a set of DsiRNAs (10 per target) targeting mouse VEGFR-1 (Flt1) and VEGFR-2 (Kdr) in 2'-O-methyl modified form on the antisense strand for comparison of *in vitro* activity with the previous set of unmodified DsiRNAs
- The best sequence for each target will be scaled up in 2010 for the *in vivo* work. The efficacy of the DsiRNAs was evaluated at WEIZMANN
- In order to track/evaluate nanoparticle delivery formulations by *in vivo* bioimaging IDT synthesized a modified EGFP DsiRNA with the LI-COR IRdye 800CW covalently linked to an aminolinker on the 5'-end of the antisense strand



Targeted Delivery of Nanomedicine



Work Package 9: Application to Cancer

Synthesis and characterization of integrin receptor-targeted bioconjugates

(IDT, MARBURG, UU, WEIZMANN)

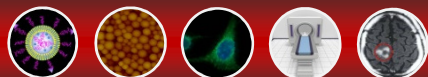
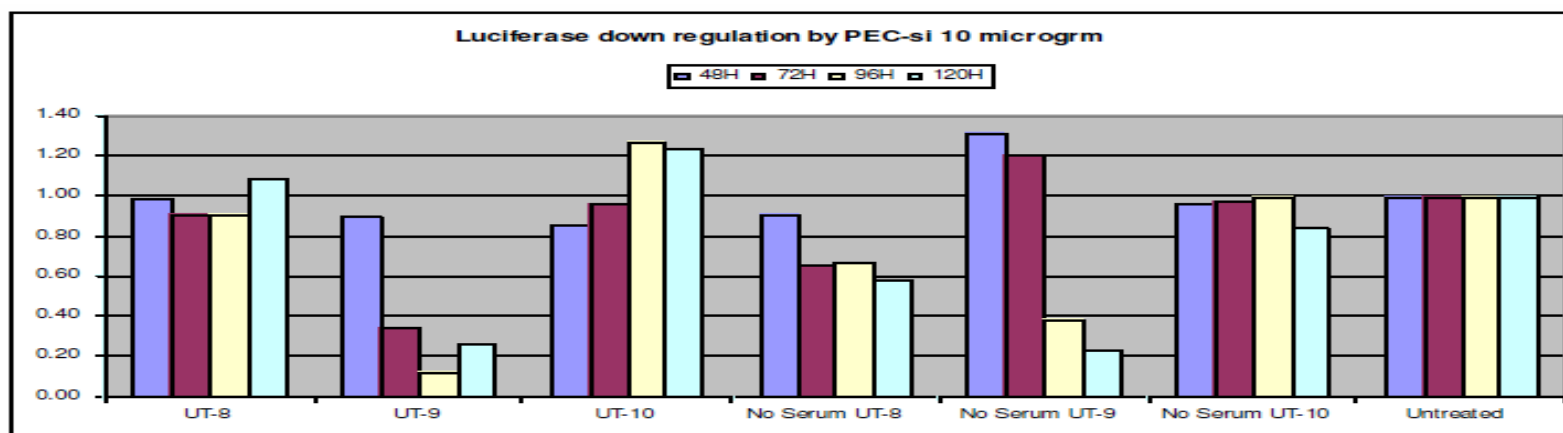
- Polyelectrolyte complexes (PEC) were prepared by self-assembly of hydrophilic polycation (poly(L-lysine) grafted with polyHPMA (GPL1)) with siRNA in aqueous 0.01M NaCl
- *In vitro* experiments of luciferase down regulation used anti Luc siRNA on MDA-MB-231-Luc-RFP human epithelial breast adenocarcinoma, that express avb3
- RFP signal used to assess cells' viability

Complexes used:

UT-8: PEC's of siRNA/GPL1; hydrodynamic radius (R_H) 40 nm, MW 6×10^5 g/mol.

UT-9: PEC's coated with reductively removable polymer LK85; R_H 49 nm; MW 9×10^6 g/mol.

UT-10: PEC's coated with reductively removable polymer LK85 and targeted with cyclic RGD; R_H 47 nm; MW 7×10^6 g/mol.



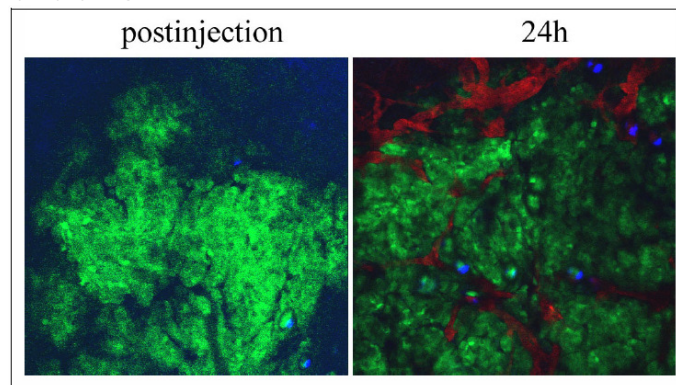
Targeted Delivery of Nanomedicine



Work Package 9: Application to Cancer

USPIO targeted imaging of ovarian carcinoma (WEIZMANN, GUERBET)

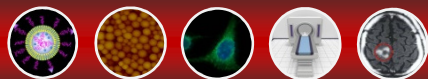
- A common cancer-targeting ligand is folic acid (FA), which targets FA receptors (FAR) that are overexpressed in several human carcinomas
- Despite proof of concept of FAR targeting, the first-generation folate targeted-USPIO (P1133) was unsuitable for clinical evaluation
- A second generation MRI contrast agent was synthesized (P3270). It used the same USPIO platform coated with biocompatible PEG and functionalized with a higher percentage of folate moiety
- Passively targeted P904 showed tumour stroma uptake through infiltration of phagocytic cells



Two photon imaging of P904-rhodamine distribution (blue) inside MLS tumour (red), and blood vessels (DiD, red). The images were acquired immediately after P904-rhodamine administration and 24h later by two-photon microscopy (2PM, Zeiss LSM 510 META NLO).

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

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



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Work Package 10: Preclinical Toxicology

Objectives

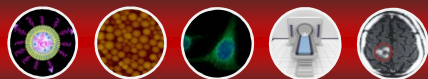
- Assess the safety risks of selected prototype nanomedicines and provide guidance in the selection process of the final products
- Assess the toxicology aspects of the nanomedicines developed in MEDITRANS in conjunction with NANOSAFE 2 and other EC projects

Work plan and conclusions from the third year

- In 2009, 4 nanomedicines were selected for WP10
- The toxicological evaluation studies will be performed by MEDITRANS partners in 2010

WP10 Leader: CEA Dr Sophie Giraud (sophie.giraud@cea.fr)

WP10 Participants: CEA, UU, BRACCO, GUERBET



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Work Package 11: Industrial Exploitation

Objectives

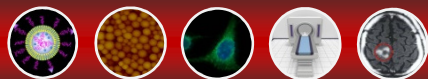
- To convert academic concepts into products
- To guide the prototype carrier systems through the predevelopment phase

Work plan and conclusions from the third year

- In 2009, 4 nanomedicines were selected for WP11
- The industrial exploitation activities will be performed by MEDITRANS partners in 2010

WP11 Leader: UU Prof. Dr Gert Storm (g.storm@uu.nl)

WP11 Participants: UU, BRACCO, GUERBET



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Work Package 12: Training

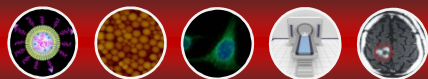
Objectives

- To provide advanced drug delivery courses for partners
- To develop the MEDITRANS website as a platform for education and training, and to facilitate the exchange of MEDITRANS' young scientists
- To provide access for MEDITRANS' 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 and conclusions from the third year

Advanced drug delivery courses

- The 'MRI Technologies for Drug Delivery' course was held on 29th March 2009 at the Weizmann Institute of Science
- The third course will be held as part of the 8th International Conference and Workshop on Biological Barriers, Nanotoxicology and Nanomedicine at Saarland University
- It is advertised on the MEDITRANS website (see www.meditrans-ip.net)
- MEDITRANS partners are encouraged to participate in other existing courses



www.meditrans-ip.net

Targeted Delivery of Nanomedicine



Work Package 12: Training

Internet-based training and a forum for scientific exchange

- The website training page received >1300 hits in 2009

Training MEDITRANS' and other young scientists

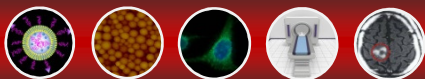
- Organised *via* the MEDITRANS' website, young scientists have been on exchange to other MEDITRANS partner's laboratories for intensive training
- Exchange reports have been prepared

Partner participation in the GALENOS-Network

- Galenos activities were promoted, graduate students applied for a Galenos Euro PhD, and 3 MEDITRANS students have been awarded Galenos Euro PhDs

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

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



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Work Package 13: Dissemination

Objectives

- To demonstrate non-confidential technologies to MEDITRANS' 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

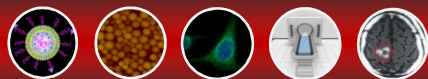
Work plan and conclusions from the third year

Demonstration of non-confidential technologies

- In 2010, MEDITRANS-developed non-confidential technologies will be demonstrated to MEDITRANS' partners and to other interested groups

Website development

- The MEDITRANS website www.meditrans-ip.net contains the latest information about MEDITRANS
- The MEDITRANS Contacts Database was used to invite key stakeholders to EuroNanoMedicine 2009



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Targeted Delivery of Nanomedicine



Work Package 13: Dissemination

Project promotion

- The outputs from MEDITRANS continue to be disseminated widely *via* partners at conferences, symposia, workshops *etc.*, and *via* the website and glossy promotional leaflets

Technology transfer workshop

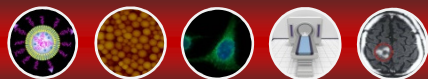
- The first MEDITRANS technology transfer workshop was combined with the EuroNanoMedicine Conference (Bled, Slovenia, 28th–30th September 2009)
- It was jointly organised by the three IPs, MEDITRANS, NANOEAR and NANOBIOPHARMACEUTICS
- The conference transferred interesting results and was well attended (see www.dechema.de/euronanomedicine2009.html)

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

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

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: The MRI brain image is from: Dousset *et al.* (2006). *American Journal of NeuroRadiology* **27**: 1000-1005.



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Targeted Delivery of Nanomedicine



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www.charite.de

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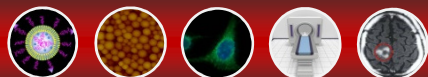
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