Executive Summary:

The demographic changes in Europe towards an aging society will coincide with increasing morbidity of the population. European citizens need improved access to state-of-the-art medical care especially in oncology and cardiology, while keeping expenditures on healthcare affordable. New therapeutic options such as externally triggered local drug release at the diseased area hold promise to solve urgent medical needs: improved treatment with reduced side effects, fewer burdens to the patient and faster recovery after intervention.

Nanomedicine, the application of nanomaterials and nanotechnology to healthcare, will enable breakthroughs in clinical practice.

SONODRUGS addressed clinical needs by developing novel drug delivery technologies for localized treatment of cardiovascular disease and cancer. Within SONODRUGS drug delivery concepts have been developed where drug release can be triggered by focused ultrasound induced pressure or temperature stimuli within the diseased tissue. New drug loaded nanocarriers have been designed for tailored drug delivery systems that respond to either of the two stimuli. Medical imaging, i.e. magnetic resonance imaging and ultrasound imaging is used to guide, follow and quantify the drug delivery process. Therapy efficacy using different drug delivery systems has been assessed in vitro and subsequently in preclinical studies. Starting from research on a broad range of materials and drugs, two nanocarriers have been finally selected, optimized, produced on lab-scale and thoroughly assessed in combination with image-guided delivery tools and methods.

SONODRUGS combined expertise in materials research (Philips Research Eindhoven, Eindhoven University of Technology, Ghent University, Nanobiotix, Aalto University); material production (Nanobiotix, Lipoid); clinical knowledge in oncology (University of Tours, University of Cyprus) and cardiology (University of Muenster); in vitro and preclinical validation (University of Tours, Erasmus Medical Center, University of Cyprus, University of Muenster, University of Bordeaux/University Medical Centre Utrecht, Philips Research Eindhoven, Eindhoven University of Technology); research on imaging techniques (University of Cyprus, Philips Healthcare, Philips Research Hamburg, University Bordeaux/University Medical Centre Utrecht, Philips Research Eindhoven); and pharmacokinetics, toxicology and biodistribution (University of London, Eindhoven University of Technology).

The following results have been achieved:
- Four concepts for ultrasound-mediated drug delivery have been explored.
- Six new nanocarrier systems for pressure and temperature activation have been developed, and validated in vitro.
- Preclinical proof-of-concept for local delivery to tumours of anti-cancer drugs encapsulated in liposomes, for local targeted delivery of compounds that without US activation would not reach their molecular targets, and for a novel combined pressure + temperature activated approach.
- Increased therapeutic effect in preclinical studies with the anti-cancer drug doxorubicin and with RNA-based compounds against cardiovascular diseases.
- Fundamental understanding of the drug release process, mechanism of action of ultrasound activation, and the relationship between material properties, release kinetics and the effect of ultrasound.

- Significant advancements towards improved and expanded medical treatment options in oncology and cardiology by providing image control for externally triggered drug release.
- 8 patents have resulted from the project, on nanocarriers, protocols, and methods for therapy monitoring.

Project Context and Objectives:

The objective of SONODRUGS was to develop new nanoparticulate drug carriers that can be activated for local drug release using focused ultrasound. Medical imaging i.e. MRI and/or ultrasound imaging is used to guide and quantify the drug release.

The focus was to develop and evaluate two drug delivery concepts:
- Nanocarriers activated by ultrasound-induced pressure stimuli, such as lipid and polymer shell based nanocarriers.
- Nanocarriers activated by ultrasound-induced temperature changes in the target tissue, such as liposomes and phase-converting liquids.

The nanocarriers are modified with contrast and imaging agents to facilitate imaging of the therapeutic intervention and the drug release process (WP1). Drug delivery is first tested in vitro (WP2) before proceeding to in vivo studies (WP3, WP4). The change in the drug biodistribution that is induced by ultrasound treatment can be quantified using in vivo imaging, and should be linked to therapy efficacy.

Next to the development of new nanocarriers, SONODRUGS developed methods for ultrasound triggering and image-guiding of the therapy (WP5). While new drug delivery systems were under development in WP1, those methods were already investigated and tested by co-administering ultrasound contrast agents (i.e. microbubbles) approved for human use together with existing drug formulations (WP2-5). The changes in therapeutic efficacy of the drug upon ultrasound activation were studied. Ultrasound induced oscillations of these types of bubbles had been shown to open transient pores in endothelial cells or cell membranes that allow more efficient drug uptake. It further allows a first assessment of ultrasound induced changes in drug uptake by cells in vitro and in vivo. The newly developed methods were in a later stage of the project applied to the SONODRUGS materials.

SONODRUGS has explored 4 different treatment concepts for ultrasound mediated drug delivery:
- Microbubbles for co-administration. Microbubbles are small (only a few micrometers) gas bubbles, stabilized by an outer shell. Microbubbles are responsive to ultrasound (US) and are therefore used as contrast agents in diagnostic applications, but at higher US intensities they implode. Such microbubbles are injected together with the drug and upon US activation implode inside the blood vessels of the targeted organ. Hereby the blood vessels are made more porous, but only locally and temporarily. This is named sonoporation, and the drug in circulation in the bloodstream will then enter the target tissue at a higher concentration.
- Drug-loaded microbubbles. Liposomes, usually 100 to 200 nanometres, can be loaded with drugs and attached to microbubbles. These nanocarriers are injected, and upon US activation and bursting of the microbubbles, sonoporation and also local release of the drug occurs. Drugs reach the target tissue at higher concentrations, and exposure of the rest of the body to the drug is limited.
- Hyperthermia induced drug delivery. US at even higher intensities can be used to cause localized controlled hyperthermia, a mild heating of the target tissue to approx. 42°C. For this, high intensity focussed ultrasound (HIFU) is used. HIFU is combined with novel temperature-sensitive liposomes engineered to release the loaded drug upon mild heating. The liposomes are injected, circulate in the body, and when they reach the heated target tissue, release the drug. The effect is that

drugs reach the target tissue at higher concentrations, and exposure of the rest of the body to the drug is limited. The liposomes can be loaded with drugs and MR contrast agents such as gadolinium or iron oxides for image guidance of drug release.
- Combined or 2-step approach. First microbubbles are imploded by US to cause sonoporation at the target site. Then temperature-sensitive liposomes are injected and the target tissue is heated to induce local release of the drug from the liposomes. This results in a synergistic increase of exposure of target organs to the drug.

Project Results:

Overall overview

Nanocarriers have been developed for ultrasound mediated drug delivery: Polymer microbubbles for co-administration, drug-loaded liposomes attached to lipid microbubbles for US-pressure induced drug delivery, and liposomes (Gd-TSL, magnetoliposomes, lipid-peptide hybrids, optimized conventional formulations) for hyperthermia-induced drug delivery.

First, these nanocarriers were tested in vitro. For most systems toxicity in cell culture was found to be low or absent, the nanocarriers were suitable for tracking and imaging, and the nanocarriers could be triggered by US to release drug and/or enhance cellular drug uptake. Moreover, selected nanocarriers underwent a full acoustic characterization, and the mechanism of action for sonoporation and drug release from drug-loaded nanocarriers was investigated.

Promising nanocarriers were then tested in vivo. For most the blood circulation time was suitable for the envisioned applications. Clearance occurred by urine, liver and spleen without unwanted accumulation in sensitive organs. In vivo toxicity was analysed by histology and no unacceptable effects were found. Excretion studies showed also rapid clearance of drug, but the slow excretion of co-encapsulated MR contrast agents may need further investigation. Studies by SPECT/CT imaging and organ biodistribution showed very nicely the biodistribution of the nanocarriers, and the enhanced uptake in target organs by US triggering of the nanocarriers.

Feedback from the in vitro and in vivo studies was used to optimize the formulations of the developed nanocarriers. A large body of knowledge has now been build up for formulation optimization. In the meantime, a clinical MR-HIFU ablation device had been altered for preclinical hyperthermia studies. Methods for in vivo tracking and triggering of the nanocarriers had been implemented on this device, and also on devices for pressure-mediated drug delivery. Moreover, methods had been established to monitor drug release during treatment. During these activities, a new approach for US mediated drug delivery was found and evaluated, the combined or 2-step delivery.

Finally, the optimized nanocarriers and the developed methods were tested preclinically. Local drug delivery could be induced in vivo using model drugs. Preclinical proof-of-concept for enhanced drug delivery was obtained for three investigated approaches. US mediated drug delivery increased tumour uptake up to 10-fold for temperature-sensitive liposomes encapsulating the anti-cancer drug doxorubicin. Moreover, the level of increase could be predicted by monitoring the release of the co-encapsulated MR contrast agent during treatment. Co-administration showed a low enhanced uptake of the liposomal drug Caelyx, but it did enable enhanced uptake of fluorescent nanoparticles and siRNA compounds in tumour and liver tissue, and radiolabelled BSA and plasmid DNA in skeletal muscle. The combined or 2-step approach enabled high tumour uptake of a model drug that otherwise cannot reach its intracellular target.

Subsequent preclinical therapeutic efficacy studies showed that tumour growth is reduced up to 3-fold fold by MR-HIFU treatment with temperature-sensitive liposomes encapsulating doxorubicin. Additionally, the dose of an siRNA compound necessary to reduce expression of a gene involved in LDL-cholesterol production was reduced 100-fold by US

mediated drug delivery with co-administration of polymer microbubbles. Thus we have obtained proof-of-concept for therapeutic efficacy in two investigated approaches.

Our results have led to 8 patents, 40+ publications in peer-reviewed journals, 100+ presentations at scientific conferences and 3 PhD theses so far (with 10 more to come). Moreover, we have organized a conference on image-guided US mediated drug delivery, and methods and techniques have been taught to researchers from within SONODRUGS and external in three hands-on trainings. Finally, the work is continued in several follow-up projects which are ongoing or have been applied for.

Overall conclusion

SONODRUGS has been a highly interdisciplinary project combining chemistry and technology, diagnosis and therapy, and called on the expertise of academic partners, university hospitals, small and medium-sized entreprises (SME) and large industry. The project has resulted in eight patents on nanocarriers, hardware and methods, 44 publications in high-ranking journals and over 100 presentations at international conferences. During the course of the project, three PhD trajectories have been finished successfully, two PhD theses are in preparation, and at least 8 more are in the pipeline.

Thanks to the team's dedicated work – both fundamental research and applied science – significant advancements in the development of novel treatment methods have been made. A body of knowledge has been built up, and young European scientists are now experts in this field. This increases the potential for healthcare innovations by European academic institutions, hospitals, and industries, backed by patents on several key findings. Concepts have been successfully tested in preclinical studies for the delivery of the anti-cancer drug doxorubicin and RNA-based compounds against cardiovascular diseases. Extrapolating these results, the platform can potentially be used for many different drugs. Perhaps more importantly, however, significant steps have been made to one day provide patients with access to treatments with higher efficacy and less side effects, which may improve their quality-of-life and reduce the debilitating effects and costs of these dreadful diseases.

Although significant progress has been made, the final goal has not been reached yet. Fortunately, the collaborations between SONODRUGS partners will be continued at the end of the project. Several fruitful collaborations have been initiated or strengthened in the past four years, and these will remain active. Illustrative of this are the continued collaborations between some of the partners in new projects that are currently running as well as new applications.

Following is a description of the most important results per workpackage:

WP1: Development of nanocarriers

The objective of WP1 involved the design and synthesis of novel materials for ultrasound-mediated drug delivery. In close collaboration with the other workpackages, these nanocarriers have been optimized in terms of size, drug loading, in vitro toxicity and in vivo applicability for enhanced drug delivery.

The work carried out in WP1 has been divided over the following tasks:
- Task 1.1: Nanocarrier development
- Task 1.2: Drug loading of nanocarriers

- Task 1.3: Optimization of nanocarrier formulation
- Task 1.4: In vitro toxicity in the absence of US
- Task 1.5: Scale-up
- Task 1.6. Final evaluation

WP1 was headed by Philips Research Eindhoven (PRE); WP leader: Dr. Marcel Bφhmer succeeded by Dr. Sander Langereis. Participating partners were: HBBG/AALTO, NBTX, TUE, UGent, EMC, Lipoid, and ULSOP.

Results - innovations
New strategies have been explored for US mediated drug delivery based on either pressure- or temperature-sensitive materials. Novel pressure-sensitive systems have been designed for drug delivery based on: i) the co-administration of polymer-shelled microbubbles and drugs (PRE); ii) drugs incorporated in the oil core of partially-filled microbubbles (PRE, EMC); iii) lipid-shelled microbubbles decorated with drug loaded liposomes (UGent). Image guidance of therapy is based on diagnostic US.

In addition, temperature-sensitive materials have been developed that release encapsulated drugs at mild hyperthermia (42°C). For instance, temperature-sensitive liposomes (TSLs) based on lysolipids, edelfosine, peptides, and asymmetric phosphatidylcholines have been developed with tuneable release properties (HBBG/AALTO, PRE, TUE, ULSOP). Moreover, TSLs co-encapsulating drugs and MRI contrast agents have been explored for image-guided drug delivery (NBTX, PRE, TUE). At temperatures close to the melting phase transition temperature (Tm) of the lipid membrane, the drug (e.g. doxorubicin) and the MRI contrast agent are released, e.g. [Gd(hpdo3a)(H2O)] or ultra-small superparamagnetic iron oxides (USPIOS). The observed MRI contrast enhancement at temperatures near the Tm of the lipid bilayer allows one to monitor drug release, due to the co-release of the encapsulated MRI probes.

The cytotoxicity of materials developed in WP1 has been tested on tumour cells and endothelial cells. For the temperature-sensitive materials none of the tested materials (NBTX, PRE, TUE, ULSOP) showed toxic effects on tumour cells with the exception of edelfosine-based TSLs (HBBG/AALTO). However, doxorubicin loading does introduce an adverse effect on cell viability. For the polymer microbubbles and the drug-loaded liposome lipid microbubbles no toxic effects on cell viability have been observed.

HBBG/AALTO: Temperature-sensitive liposomal formulations based on LTSL liposomes with the bio-active lipid edelfosine added have been developed. They displayed an improved profile for drug leakage and triggered release compared to standard lysolipid-based TSLs. Moreover, a rational and concise protocol has been implemented for the development and characterization of new temperature-sensitive liposomes formulations enabling the minimization of the leakage of doxorubicin at physiological conditions, while maximizing the doxorubicin release rate in hyperthermic areas.

NBTX: A series of novel doxorubicin-loaded magnetoliposomes has been explored. The surface treatment of the iron oxide nanoparticles was essential for the co-encapsulation of doxorubicin and iron oxide nanoparticles in the core of the liposomes. An appropriate surface coating of iron oxide nanoparticles was established, with sufficient steric hindrance to avoid doxorubicin adsorption onto the nanoparticles surface and sufficient electrostatic charges to avoid nanoparticles aggregation.

PRE: Polymer microbubbles with poly(L-lactide)-poly(fluorooctane) as a biocompatible polymer have been developed. Polymer microbubbles retain their acoustic properties for US imaging and activation longer compared to lipid-shelled microbubbles. Partially oil-filled systems containing paclitaxel remain acoustically active. Calculations of the drug payload based on the maximum number of microbubbles that can be administered clinically and the circulation time of the microbubbles, revealed that delivery of a sufficient dose of drug to a target region was very challenging. Therefore, this approach has been abandoned, and the microbubbles have been used for co-administration applications. In addition, TSLs based on asymmetric phosphatidyl-cholines have been developed. The incorporation of these novel lipids in the lipid bilayer allows fine-tuning of the melting phase transition temperature between 38.7 and 39.6°C.

TUE: The co-release of doxorubicin and MRI contrast agents from the lumen of temperature-sensitive liposomes allows for MR image-guided doxorubicin delivery by probing the change in the longitudinal relaxation time. The TTSL formulation has been selected as the most promising candidate for in vivo US-mediated drug delivery.

UGent: Drug-loaded microbubbles were obtained by attaching drug-loaded liposomes to lipid microbubbles. Initially, non-covalent loading of biotin-functionalized liposomes encapsulating doxorubicin to the avidin-functionalized surface of lipid-shelled microbubbles was used. After receiving feedback from the other WPs, the formulation of the liposome loaded microbubbles was altered into a sterile, storable formulation, in which the immunogenic avidin-biotin systems used for non-covalent coupling were replaced by thiol-maleimide conjugation chemistry.

ULSOP/UCL: Leucine zipper peptides have been incorporated successfully into lipid bilayers during liposome preparation without affecting liposome morphology, size characteristics, and doxorubicin encapsulation efficiency. These peptide-modified liposomes showed a higher serum stability compared to the standard LTSL. Upon hyperthermia, these peptides undergo a conformational change inducing release of the encapsulated drugs.

In conclusion, novel strategies for temperature- and pressure sensitive materials have been developed for US-mediated drug delivery. These materials have been optimized in terms of size, drug loading, and drug release profiles. Initial in vitro toxicity assays with the nanocarriers (without drugs) on tumour cells and endothelial cells showed no toxic effects, with exception of the edelfosine-based liposomes. These novel nanocarriers have been transferred to WP2-4 for a detailed in vitro and in vivo evaluation.

WP2: In vitro concepts and evaluation

Workpackage 2 aimed to evaluate in vitro the drug nanocarriers developed in WP1, prior to subsequent in vivo testing in WP3 and 4. The evaluation concerns both pressure sensitive particles and thermosensitive particles. The effect of US on drug delivery has been studied quantitatively. US parameters such as frequency and acoustic amplitude have been investigated to achieve optimal drug release and delivery to cells. Additionally, we have explored the mechanisms by which US stimuli induce drug release from both pressure and temperature sensitive drug carriers.

The work carried out in WP2 has been distributed over five tasks:
- Task 2.1: Co-administration of approved drugs and contrast microbubbles
- Task 2.2: Quantification and optimization of drug release with nanocarriers developed in WP1
- Task 2.3: Acoustic characterization and imaging of drug loaded microbubbles
- Task 2.4: Drug release using temperature sensitive carriers
- Task 2.5: Biological activity evaluation and release mechanism

WP2 was headed by University of Tours, FR (UTours): WP leader Prof. Dr. Ayache Bouakaz. Participating partners were: EMC, UCY, UGent, and UKM.

Results - innovations

First, co-administration using registered agents was studied. We have demonstrated that under a variety of experimental conditions, an enhanced cellular uptake of a marker dye or a drug can be obtained. When US and microbubbles were applied the compounds were taken up in the cells and US parameters could be chosen such that cell viability was not impaired. In contrast, in the absence of US and/or microbubbles there was no cellular uptake of any of the tested molecules. In our tests, there was no limit with respect to the molecular weight of the compounds: small molecules up to very large compounds such as dextran with a molecular weight of 70 kDa or plasmid DNA were efficiently delivered, all at clinically acceptable US settings below 1 MPa and frequencies around 1 MHz. The results suggest that this combination is a useful tool for localized triggered drug delivery and may have applications in cancer therapy.

Besides the co-administration approach we investigated drug-loaded pressure-sensitive nanocarriers, in which the drug was incorporated in liposome nanoparticles attached to lipid-shelled microbubbles. We have shown that US can indeed be used to release the drug from the microbubble carrier. Drug release occurs when the microbubbles are destroyed with US, either as free molecules or still encapsulated in the liposomes. All US conditions that are able to destroy the microbubbles will cause efficient drug release. In addition, cell viability measurements have shown that the procedure is safe since US treatment on its own did not induce a significant loss of cell viability.

Various formulations of pressure sensitive carriers have been made within WP1. After testing in WP2, the drug release from the drug-loaded lipid microbubbles (via bound liposomes) was shown to be more efficient than from similar polymer microbubbles. Therefore, the drug loaded lipid microbubbles have been selected as the pressure sensitive drug carrier for the subsequent experiments.

In a next step, we characterized the selected pressure sensitive drug carriers using optical and acoustical approaches. To this end, high speed optical observations have been performed. The results showed that the shell elasticity of the microbubbles is not altered by the attached liposomes. However, a clear difference was found in the shell viscosity of drug loaded microbubbles that were larger than 6 µm. Acoustical characterization has also shown that the loaded microbubbles behave similarly to commercially available contrast agents. They can be efficiently imaged for diagnostic purposes by conventional US scanners with a high contrast to tissue ratio, using non-destructive modes. Moreover, at selected US settings, their destruction can be triggered under both static and flow conditions, hereby inducing the release of the payload drug. Overall, the ultrasonic features of the selected drug

loaded microbubbles are essential in designing dedicated delivery and imaging protocols.

The thermosensitive nanoparticles developed in WP1 were also characterized in vitro. A dedicated experimental set-up has been developed, based on a double compartment which mimics the in vivo situation. This set-up has been exploited to define the US parameters required to induce the necessary temperature increments to activate the nanocarriers. The results showed that US heating resulting in temperatures of 42°C and higher induces up to 90% of drug release from the nanocarriers. Such temperatures can be reached by applying clinically acceptable US settings. For the US transducer used in the set-up an acoustic pressure higher than 1.4 MPa with a duty cycle of 50% was sufficient.

Finally, we have investigated the mechanisms by which the drugs are released from the nanocarriers and the routes by which they are incorporated into the cells. For the drug-loaded lipid microbubbles, experiments using fluorescently labelled nanocarriers indicate that three different mechanisms might be responsible for drug release: movement of fluorescent material over the bubble surface and rearrangement of the lipids; release of small vesicles (several hundreds of nanometres); and the release of a bulk of particles which are too small to be resolved individually, observed as a mist emerging from the microbubble surface. After release, it is likely that the drugs enter into the cells either encapsulated in the liposomes or as free molecules depending on the applied US activation. Moreover, when pressure sensitive particles are co-administered with drugs, both pore formation and endocytosis processes can take place and are likely associated with the drug uptake into the cells. For the thermosensitive nanocarriers US heating induces drug release from the carriers. The mechanism of uptake in cells does not appear to be different from the one achieved using the drug in its free form.

Conclusion

In WP2, we have evaluated in vitro the nanocarriers developed in WP1. We have demonstrated that the co-administration approach (drugs and microbubbles) provides a beneficial therapeutic effect and thus might be useful for cancer therapy.

Drug-loaded microbubbles have been characterized and their therapeutic efficacy evaluated. These drug-loaded microbubbles showed to be an efficient imaging contrast agent and their payload can be released externally and on demand through selected US parameters. Furthermore, we have shown that the therapeutic efficiency of the drug included in these liposome loaded microbubbles is significantly increased after US exposure and microbubble implosion.

Temperature-sensitive nanocarriers developed in WP1 have been investigated using a dedicated setup. Our results showed that suitable US scanning conditions can be defined to achieve the desired temperature elevation and thus drug release from the thermosensitive carriers.
The data from WP2 have been used to optimize the nanocarriers in WP1 and selected formulations were transferred to WP3 for further in vivo testing.

WP3: In vivo biodistribution and pharmacokinetics

SONODRUGS WP3 focused on the in vivo biodistribution behaviour of the temperature-sensitive and pressure-sensitive nanocarriers that were developed by SONODRUGS partners. The work conducted in this WP studied thoroughly the behaviour of the carriers as well as the encapsulated materials (therapeutic and diagnostic agents). The biodistribution, blood half-life time, toxicity and excretion pathways of the nanocarriers were determined in healthy and tumour-bearing rodents, with or without activation by US pressure waves or hyperthermia.

The tasks within WP3 are summarised as:
- Task 3.1: Tissue distribution of radioactively labelled nanocarriers
- Task 3.2: Real-time molecular imaging of co-administration
- Task 3.3: Real-time molecular imaging of temperature sensitive nanocarriers
- Task 3.4: Blood circulation and pharmacokinetic studies of nanocarriers including pharmacokinetic modelling
- Task 3.5: Metabolic excretion of nanocarriers and histological analysis of organs at risk
- Task 3.6: Comparative studies of the effect of US on pharmacokinetics and biodistribution, including gene delivery

The work conducted in this work package is a result of fruitful collaborations between The School of Pharmacy University of London, UK (ULSOP, work package leader Prof.Dr. Kostas Kostarelos); TUE and UKM.

Results - innovations

Temperature-sensitive and pressure-sensitive nanocarriers that showed promising results in WP1 and WP2 were transferred to WP3 for in vivo testing.

The first system studied was the pressure-sensitive polymer microbubbles. The polymer microbubbles were labelled with the radioactive gamma isotope Indium-111 and administered preclinically. The microbubbles were cleared from the blood with a half-life of 4 min, and accumulated predominately in the liver and spleen as shown by non-invasive SPECT/CT imaging. Microbubbles can be used for co-administration and as drug-loaded nanocarriers. For the polymer microbubbles the relatively short blood circulation is the main challenge to application as a drug-loaded delivery system. However, in WP2 was shown that these microbubbles are effective in co-administration in vitro. In WP3 we tested this observation in vivo. Co-injection in combination with US consistently increased tissue uptake of radiolabelled albumin and fluorescently labelled nanoparticles in skeletal muscles and tumour tissues. The enhancement in drug uptake efficiency was highest just after treatment and then decreased with a half-life of about 20 min for the investigated compound. These findings are very promising for applications in the field of oncology and gene delivery, where hydrophilic drugs and nucleic acids exhibit a low cellular uptake in the target tissues.

We also studied the biodistribution of a variety of temperature-sensitive liposomes (TSL). The focus was on the nanocarrier platform in which the drug doxorubicin and a gadolinium-based MRI contrast agent were co-encapsulated in several liposome types, resulting in an image guided drug delivery system. The blood kinetics of both the liposomes and the encapsulated drug was evaluated. Blood circulation time of all tested liposomes was highly affected by the size of the liposomes and the formulation composition. A smaller size (less than100 nm) and a higher phase transition temperature of the lipid bilayer prolong blood circulation of the nanocarrier. The latter parameter also directly

influenced the serum stability of the nanocarriers to retain the encapsulated drug and contrast agents. Tissue distribution of the nanocarriers and the encapsulated drug was shown to depend on liposome size, lipid composition and their stability in vivo. Formulations with low stability were cleared faster from the blood and accumulated in the liver, while free drug and contrast agents were excreted via the kidney. Overall, a low accumulation of the nanocarriers was observed in the liver and spleen of 10-20% of the injected material, which was gradually excreted from the body.

The effect of mild localized hyperthermia (41-42°C) on biodistribution of the TSLs was evaluated by warming up the tumour on the hind limb in a water bath during administration. The liposome blood profile and organ biodistribution were not affected. On the other hand, much more liposomes and drug were delivered to the heated tumours. This enhancement in tumour accumulation is caused by a local drug release from TSL in response to hyperthermia, augmented by an increase in tumour blood flow and vascular permeability due to the hyperthermia. Results in WP2 showed that US can induce hyperthermia and cause efficient drug release from TSL in vitro. This observation was tested in WP3 in vivo using high-intensity focused ultrasound (HIFU). Non-invasive SPECT/CT imaging of radiolabelled TSLs that had been activated by HIFU showed a much higher tumour accumulation of the nanocarriers, agreeing with the results obtained with water bath induced hyperthermia. Non-invasive MR imaging showed release of contrast agent and drug, and histology of the HIFU-treated tumours confirmed the higher liposomal accumulation and showed a more complete tumour penetration of the liposomes and doxorubicin.

The pharmacokinetic data from the WP3 in vivo experiments was used for modelling of the behaviour of the TSLs. A pharmacokinetic model was developed that includes liposome blood circulation, drug leakage, liposome organ uptake, and tumour accumulation in the presence and absence of hyperthermia.

The histological examination and metabolic excretion of the different TSL formulations and the polymer microbubbles were also studied in preclinical models. The rate of contrast agent excretion was shown to depend on stability of the liposome, with agents from stable formulations taking longer to be excreted from the body. Despite the slow degradation and excretion of TSL from tissues, no significant toxicity was observed in the major organs studied up to 2 months by microscopy histological examination. A similar slow excretion but no toxic effects was observed with the polymeric microbubbles.

Conclusion

Extensive work has been carried out in WP3 to label the polymer microbubbles and several TSL with radioactivity and evaluate their pharmacokinetics and serum stability in vivo. Following systemic administration, polymeric microbubbles showed short blood circulation ($t_{1/2}$ shorter than 5 min). Co-injection of microbubbles and therapeutic agent in the presence of US significantly improved drug accumulation in US-treated tissues, which has a "temporal window" with a half-life of approximately 20 min for the compound and US settings tested.

TSL behaviour (blood kinetic, serum stability and organ uptake) in vivo depended on liposome size as well as lipid composition. Two hyperthermia set-ups were successfully established to induce local hyperthermia in vivo; water bath and HIFU. Local hyperthermia had a negligible effect on

liposome pharmacokinetics to the major organs. On the other hand, hyperthermia dramatically enhanced accumulation of liposomes and drug in tumour as well as penetration within the tumour mass. As a proof of concept we showed that MR-HIFU is a feasible tool to allow tracking the biodistribution and drug release from TSL encapsulating drug and MR contrast agent. Finally, no toxicity was observed with any of the tested materials even up to 2 months after systemic administration.

Materials have been optimized in WP1 based on these results, and selected materials were transferred to WP4 to assess delivery and therapeutic efficacy in vivo.

WP4: In vivo evaluation

The objectives for WP4 were to preclinically validate the newly developed nanocarriers (pressure-sensitive and thermosensitive) within SONODRUGS. Studies to test methods for controlled drug release, quantify drug release and uptake, and evaluate therapeutic efficacy were the main objectives.

The tasks within WP4 are summarized as:

- Task 4.1: Treatment efficacy evaluation of co-administration with commercial microbubbles
- Task 4.2: US guided local drug release
- Task 4.3: Quantification of local drug release and uptake
- Task 4.4: Evaluation of treatment efficacy using SONODRUGS nanocarriers

WP leader was Prof.dr.ir. Nico de Jong from EMC. Participating partners were PRE, TUE, UMCU, UKM, and UCY.

Results - innovations

Pressure sensitive nanocarriers

The polymer microbubbles that were developed in WP1 for co-administration were validated preclinically. First, microbubbles were co-injected with dyes that under normal circumstances remain in the blood stream. US treatment resulted in an efficient delivery to muscle and liver tissue, but less to tumour tissue. Next, the nanoparticle drug Caelyx®, a liposomal formulation of doxorubicin, was tested. The accumulation in tumours is already quite efficient, and this could not be improved by co-administration treatment. An enhanced anti-tumour therapeutic effect was also not detected. After these negative results with small molecules and liposomes, focus was shifted to co-administration of RNA based therapeutics. In contrast, successful improvement was shown in preclinical tests for siRNA delivery. An RNAi compound that lowers LDL cholesterol formation was efficiently delivered to liver cells by US treatment. In this case, US mediated drug delivery was able to lower the effective dose of the compound by a factor of 100.

Lipid microbubbles were also investigated preclinically for co-administration. The influence of variable US pulsing schemes on the delivery of the model drug FITC dextran was evaluated in an in vivo tumour model. Focused US at a high pressure of 2 MPa combined with co-injection of lipid microbubbles and FITC-dextran enhanced delivery into tumour tissue 14-fold in comparison to US exposure without administration of microbubbles (51.3±9.0% vs. 3.7±0.5%). A dual pulse consisting of a 0.4 MPa low-power pulse followed subsequently by a 4 MPa high-power pulse increased the FITC dextran delivery also significantly (18.0±2.8%). The dual pulse caused less damage as shown by the lower number of apoptotic cells (6.1±5.5% for 2 MPa vs. 0% for 0.4/4 MPa). Histologic analysis of HE stained tumour sections revealed no signs of morphologic differences or haemorrhages in all treated groups.

Thermosensitive nanocarriers

In WP1 several temperature-sensitive liposomes have been developed. Of these, especially the TSL incorporating doxorubicin and the MR contrast agent Gd-DTPA were investigated in vivo. The MR-HIFU combined imaging and therapy system was used to induce drug delivery under MR image guidance. First, an in vivo study was performed to study drug release and uptake in the target tissue. The release of doxorubicin and the MR contrast agent was investigated in a 9L glioma tumour model at mild hyperthermia (T = 42°C). A clinical Philips Sonalleve MR-HIFU system was equipped with a binary feedback loop for controlled heating of the tumour between 41°C and 42°C using a 4 mm volumetric treatment-cell (acoustic power: 8 W; US frequency: 1.44 MHz). A specific small animal system add-on was employed to enable preclinical MR-HIFU treatment with the clinical Sonalleve MR-HIFU platform. The dedicated system had a HIFU compatible partially submerged 4-channel SENSE MR coil placed above the transducer (WP5). After preparation and positioning, the tumour was sonicated for two consecutive 15 min intervals. Upon reaching the desired temperature of 42°C in the target area, the TSLs were administrated at a dose of 5 mg/kg bodyweight. A control group was injected with TSLs without applying sonication. The MR-HIFU system was able to maintain the temperature inside the tumour between 41°C and 42°C during the complete hyperthermia period. From the T1 maps (using a Look-Locker sequence) before and after the treatment of the HIFU induced hyperthermia group and the control group (no HIFU) it is clearly visible that HIFU activation of the nanocarriers causes a strong change in MR signal. In addition, the amount of doxorubicin and Gd that had accumulated in the tumours was analyzed, and a linear relation was found between drug uptake and the contrast agent release. This allows for non-invasive monitoring of the treatment via MR imaging of the contrast agent. Moreover, the HIFU induced drug release was very successful: drug concentration in the HIFU heated tumours was 9 times higher compared to the control group without heating.

A similar protocol was used to perform the therapeutic efficacy study. A preclinical R1 rhabdomyosarcoma tumour model was treated with MR-HIFU hyperthermia and TSL encapsulating doxorubicin and contrast agent. Tumour growth was delayed by a factor of 3 compared to control treatments such as TSL without HIFU, non-temperature sensitive nanocarriers and the conventional free formulation of doxorubicin. The anti-cancer effect was correlated to the MR contrast monitoring value during treatment.

Combined or 2-step approach

Finally, preclinical evaluation of the 2-step approach was performed using TOPO-3 as a model drug. This dye is cell-impermeable and is highly fluorescent only when bound to DNA, an intracellular target. In a preclinical tumour model sonoporation was induced by systemic administration of clinically approved microbubbles with focused US, followed by hyperthermia and injection of TSL encapsulating TO-PRO-3. A clear fluorescent signal from the tumour was obtained by in vivo optical imaging after the two consecutive steps, but not after only one of the steps, showing that the combined action is required for intracellular delivery of the model drug.

Conclusion

Preclinical proof-of concept was obtained for delivery of drugs to tumours for all three in vivo tested concepts: co-administration, hyperthermia and the combined approach. Moreover, proof-of-concept of therapeutic efficacy was obtained for delivery of siRNA to the liver for

cardiovascular applications, and tumour treatment by doxorubicin from TSLs. The latter is a prime example of image guided triggered drug delivery as monitoring of the drug presence and quantification of release by MR was highly predictive of therapy outcome.

All methods appear to have high potential and are suited for continued studies. For co-administration the number of cycles in combination with the acoustic pressure appears to be a critical factor influencing the effectiveness of the delivery strategy, but damage to the treated area is a point of caution. For TSLs local high increases in drug accumulation and significant slowdown of tumour proliferation have been reached.

WP5: Method development

The SONODRUGS project is dedicated to the development of nanocarriers triggered for local drug release using focused US. Within this overall framework, the general objective of workpackage 5 is the development of methods for focused US triggered local drug delivery and image guidance methods that can be used for the evaluation of the efficacy of drug delivery, and in particular for the evaluation of pressure and temperature sensitive nanocarriers developed within the SONODRUGS project.

The tasks of this WP were:
- Task 5.1: Development of MR-HIFU methods for temperature-sensitive systems from WP1
- Task 5.2: Modelling and measuring the US field produced by the focused US transducers
- Task 5.3: Development of methods for co-administration with existing microbubbles
- Task 5.4: Same, for microbubbles developed within SONODRUGS
- Task 5.5: Real-time MR monitoring of drug release and drug distribution
- Task 5.6: Development of a modified MR-HIFU system

WP leader was Prof.dr. Chrit Moonen from IMF/UMCU. Participating partners were PRE, PRH, UCY, and PMS.

Results - innovations

Image guidance is expected to improve the spatial control of triggered drug delivery, as well as provide an early prediction of outcome. Adapted US delivery schemes and precise knowledge of spatio-temporal aspects of drug release and delivery are crucial for successful implementation and validation of the methods.

Methods have been developed for monitoring and activation for both the pressure-sensitive and the temperature-sensitive approach. First, measurements and modelling of the US field produced by the US transducers in the SONODRUGS project have led to a thorough understanding and validation of the pressure field generated in and around the focal point of the US beam.

For the co-administration approach, methods have been developed to induce microbubble cavitation starting from the settings found in WP2. These methods were subsequently implemented on the device used in WP4.

For delivery via temperature-sensitive liposomes significantly more development was required. First a clinical device for HIFU ablation needed to be adapted for preclinical hyperthermia studies. In order to be able to use the existing clinical MR-HIFU systems for work in anesthetized small animals, a specific holder was made allowing to

maintain animal body temperature, to provide direct access of the US beam to the target tissue, to use anaesthesia gases, and optimal MRI signal strength by integration of multiple MRI receiver coils in the animal holder. In order to detect presence of the nanocarriers at the target tissue, MRI as well as US imaging can be used in guided HIFU triggered drug delivery. Each method has its particular advantages and disadvantages. US imaging can detect individual microbubbles, and is very fast. MRI is the only method that allows accurate temperature imaging in vivo for the necessary tight control of hyperthermia. In addition, MRI can detect the co-release of MRI contrast agents, co-encapsulated with the drugs in nanocarriers. The methods developed in this workpackage allowed simultaneous detection of co-released contrast agent and temperature mapping. Moreover, during the course of the project, a 2-step method was developed that combines US triggered sonoporation using microbubbles in combination with local release from temperature sensitive nanocarriers.

Finally, real-time monitoring of the drug delivery process and cellular uptake would be very beneficial to quantify the process of US triggered drug delivery. The co-release of MRI contrast agent allows the direct measurement of release of drugs from the nanocarriers. This way, the first indications whether the individual patient will likely benefit from the therapy will be available after one treatment session already.

In addition, optical imaging appeared very beneficial for quantification of model drug uptake by cells following sonoporation. Using optical contrast agents whose fluorescence yield is modified upon cellular uptake, we have been able to study the kinetics of transport over the cell membrane. Such methods also led to the observation that sonoporation effects on membrane permeability may last much longer than previously thought (several hours) leading to new opportunities with respect to the US protocols.

Conclusion
Methods for image-guided US drug delivery were developed, implemented and used for the in vivo evaluation that is described in WP4. Apart from the above results obtained within the SONODRUGS project, it is worthwhile to put our efforts in perspective of the worldwide research in the field. US triggered drug delivery has become a hot topic, as judged from major research funding programs by the NCI and NIBIB (NIH, USA), and papers by leading MR-HIFU research groups (Hynynen, University of Toronto; MacDannold, Harvard; Wood/Dreher, NIH). The SONODRUGS project has been a timely precursor in this field. The extensive knowledge base developed with and by the partners has put the consortium in an excellent position to build on the results, and bring this technology to the clinic.

WP6: Knowledge management
All partners participated in the knowledge management work package WP6. The work package leader was Prof.Dr. Mike Averkiou (UCY).

The objectives of WP6 were to
1) obtain maximum impact of the project results in the scientific community and in the clinical research community;
2) disseminate the technologies and experimental results via publications and conferences to researchers and equipment and pharmaceutical industry;
3) exploit the project results for the benefit of the industrial partners and the European community
4) protect IPR interests of the partners.

Activities

First, a public website (see http://www.sonodrugs.eu/ online) was set up at the beginning of the project. It serves as a communication portal to the outside world, and contains a restricted area that serves as an internal share site for the consortium.

At the end of the project, SONODRUGS resulted in 36 publications in peer-reviewed journals, with 8 more in press or in review and many more expected to come after the official end of the project. The publications cover the areas of ultrasound, molecular therapy, drug delivery, applied physics, and pharmaceutics. A complete overview of the consortium's publications is listed on the SONODRUGS web page. In addition, 3 PhD theses have resulted from the project, with 10 more expected after the end date, and many MSc theses. With regard to scientific conferences, numerous presentations at national and international meetings have been given. Many of the presentations have resulted from collaborations of two or more partners indicating strong interactions within project. Up to the time of compiling this deliverable 112 conference abstracts have been submitted as follows: 13 conference proceedings papers (includes a 2-4 page conference proceedings in addition to an abstract), 57 abstracts for oral presentations, and 42 for poster presentation have been presented. Most presentations have eventually resulted in full papers in peer reviewed journals.

To protect the intellectual property of the partners in the project, 8 patents applications have been filed to date. The patent applications resulted from collaborative work between the groups and the majority of the patents have authors belonging to two or more different groups, demonstrating good interactions between the project members.

Finally, dissemination towards the general public has been sought by four press releases, which have led to newspaper and website articles, as well as to appearances in TV and radio shows.

An external scientific advisory board (SAB) for the project has been formed with European and US experts in the fields of oncology, cardiology, and US-responsive materials, in alignment with the objectives of the project. Three half-day meetings of the consortium with the SAB have taken place during the course of the project, next to several one-on-one meetings of SAB members with partners. The consortium members prepared short presentations, which were followed by long discussions with the SAB addressing specific issues, such as translation from preclinical studies to clinical applications, the two-step delivery, the prospect of pressure sensitive materials, and co-administration.

Finally, an exploitation plan for the project output has been outlined from contributions by all partners. The industrial partners are focusing on expanding their market position in medical devices for imaging and therapy, nanocarriers systems for drug delivery, and ingredients for such. The academic partners are focusing their exploitation efforts on improving and enhancing their academic curricula, attracting high potential students, and increasing their research activities by improving their potential to attract research funds from local and international funding agencies.

Conclusion

The project has been very successful and results have been presented in 100+ conference proceedings and published in 44 manuscripts in peer-reviewed journals so far. In addition, important aspects of the intellectual property have been protected with patent applications. One key factor in all publication activity is that a large portion of the papers and patents have resulted from work from multiple sites indicating a high level of collaboration across sites and excellent team work, one of the main reasons to enter into this type of EU Large-scale Integrating Projects.

WP7: Training

The primary objective of WP7 is to disseminate knowledge on image-guided US-mediated drug delivery both within SONODRUGS amongst the partners as well as to the wider scientific and medical community via training activities related to the results of SONODRUGS.
WP7 was lead by HBBG/AALTO (Prof.Dr. Paavo Kinnunen), with partners UKM, IMF/UMCU and PRE participating.

Activities

Within SONODRUGS 4 activities have been organized, dedicated to Molecular Imaging to follow drug delivery, understanding how the innovative MRI guided HIFU method can be beneficial in the application of thermosensitive liposomes, and to fundamental understanding of molecular engineering of lipid nanosized carriers.

Training workshop on MRI-HIFU, May 2009. This course was organized by partner IMF, 6 months after the start of the project. The course lasted one week, consisting of lectures on MR and HIFU theory as well as instrumentation, combined with demonstrations and hands-on experiments. This training was set up for a small number of participants to enable direct personal contacts between the students and the lecturers. Lecture material containing also recent and unpublished results was provided prior to the course. The evaluation by the students was extremely positive in all aspects and the course can be deemed as a great success in disseminating information and enabling the students also to network.

Training course on small animal imaging, November 2010. The course was organized by the project partner UKM in cooperation with the European Institute for Molecular Imaging (EIMI), Seventh Framework Programme (FP7) project ENCITE (via WWU) and SFB 656 MoBil. The aim of this course was to convey knowledge of different imaging methods such as MRI, positron emission tomography (PET), computed tomography (CT), ultrasound and optical imaging, focussing on small animals. The training lasted one week and was available to researchers within the SONODRUGS project as well as to interested persons from external projects. The participants learned about the theoretical background and the possible applications of the imaging devices in lectures and applied their knowledge in hands-on training sessions. Experts in the different imaging technologies held lectures and tutors supervised the hands-on sessions. Each modality was covered by two hands-on sessions. One part was the practical application of the imaging method and the second session dealt with the interpretation and analysis of the acquired datasets and images. For a better comparison of all imaging modalities both an oncology and a cardiovascular model were examined. In the last hands-on session the participants were asked to analyse one modality in detail in 4-person groups and demonstrate their results in a short presentation. This was followed by a discussion of the comparability of all imaging modalities in terms of relevant medical questions: tumour or infarction size,

perfusion of tissue, applicability of imaging modality for different problems, accuracy, as well as advantages, disadvantages and limitations of the different imaging techniques.

Summer school on Molecular Engineering, June 2011. The Summer School was held in Utφ, Finland, and was organized by partner HBBG / Aalto University under the auspices of SONODRUGS, the European Science Foundation (ESF) EUROCORE program EuroMEMBRANE, Kibron Inc. and the Graduate school GSOCCB from the University of Turku, Finland. EuroMEMBRANE is of particular importance as biomembranes represent the paradigm for self-organizing structures that are found in a number of key modalities in the living cell, thus readily illustrating the possibility to copy these structural features into artificial devices by molecular engineering.

The one-week Summer School focused on molecular level on physics of lipids, their properties, organization and interactions with proteins and other biopolymers, starting from fundamental principles. Among the teachers were some of the leading pioneers in the physics of lipids and soft materials. Building on fundamental molecular and system level knowledge the students explored how biophysic principles are involved in the operation of biomembranes, their organization, physiological functions and pathological processes. Moreover, the exploitation of these fundamental principles for molecular engineering of e.g. sensors, drug delivery and medical imaging technologies was demonstrated. The Summer School comprised of lectures with significant time for free discussion. Students were encouraged to prepare a poster of their work, and some students were selected to give a 30 min talk about their work. Also, the location on a small island in the Baltic Sea increased interaction between students and lecturers improving their comprehension of presented material and allowing discussion of various scientific problems in an informal atmosphere.

Conference on US-triggered image-guided drug delivery, April 2012. In the final year of the project, SONODRUGS organized a symposium on US mediated drug delivery in general and our recent achievements. In order to reach a significant audience it was decided to partner with an existing conference. The 12th European Symposium for Controlled Drug Delivery (ESCDD) was selected on the basis of aligned aims and topic, target audience, and good experiences at previous editions. The 12th ESCDD consisted of 26 plenary lectures from pharmaceutical scientists, polymer scientists, bioengineers, biochemists, and tissue engineers from European and non-European countries, in addition to a poster session with 110 posters. There were 160 participants from 25 different countries. The conference lasted 2.5 days. The SONODRUGS session "US mediated drug delivery" took place the first afternoon. It featured 4 presentations by SONODRUGS members and 1 by a guest speaker, Dr. Bradford Wood of NIH, who has collaborations with different partners of SONODRUGS. In addition, 12 posters were presented by SONODRUGS researchers.

Conclusion

The dissemination and education activities of SONODRUGS were very successful. In high level activities knowledge and skills present and gained within SONODRUGS were shared with the partners of the project as well as with the wider scientific community.

WP8: Project management

In WP8 the project and related activities were coordinated, including interactions between the work packages and communication with the EU. The SONODRUGS Project Coordinator and leader of WP8 was Dr. Simone Vulto, succeeded by Dr. Charles Sio, both of PRE. All WP leaders participated in this WP.

Activities

The project was organized in 5 technical and 3 non-technical workpackages. The WP leaders formed the Project Management Team, which discussed by phone conference every month and 3 to 4 times per year face-to-face, usually coinciding with other SONODRUGS meetings.

Meetings of the SONODRUGS consortium were organized twice per year, each by a different partner. During these meetings, updates per WP and per partner were presented and discussed within the entire consortium, and WP meetings took place to discuss results in detail and plan the next activities.

In addition, two meetings with the Scientific Advisory Board were held to discuss progress, clinical and industrial translation and application, and next steps. Finally, two Review Meetings were held in which we discussed progress and plans with representatives of the European Commission, in June 2010 and December 2011.

Conclusion

The SONODRUGS project was a true interdisciplinary project, in which partners with many expertises, in various settings and from different countries worked together towards a single goal; to bring new therapy options for life-threatening diseases forwards. To this end, interaction and integration of workpackages and partners with diverse tasks was required. Frequent and open communication by various communication methods, complemented by face-to-face meetings, resulted in intense and fruitful collaborations. This enabled us to make significant progress in the interesting and promising yet challenging field of image-guided US mediated drug delivery.

Potential Impact:

Socio-economic impact
Local triggered release increases delivery of drugs to the sites in the body where it is needed. Hereby, effect against the disease is increased and exposure to the rest of the body (toxicity) is expected to decrease. The patient will experience a higher therapeutic effect with lower side effects. In SONODRUGS, we use ultrasound (US) as the trigger. US is well-known for diagnostic applications, it is wide-spread, easy to use and safe. The nanomedicine drug is injected, and US activates the nanomedicine drug from outside the body, causing a highly controlled and localized activation without invasive procedures. This will reduce patient burden and hospitalization. We use image guidance by the well-known diagnostic modalities of US and MRI, so that the clinician receives real-time feedback during the procedure.
Significant steps in novel oncology and cardiovascular treatment methods have been made, both on a fundamental research level as well as on applied science level. In the end healthcare in Europe will be improved. Patients may have access to treatments with higher efficacy and less side effects, which should improve their quality-of-life and reduce the debilitating effects of these dreadful diseases. Importantly, doctors may have access to new treatment methods and, overall, the costs to society of the disease may decrease.

Wider societal implications
European principal investigators have built a body of knowledge to be used to bring US-mediated delivery and related nanomedicine methods further. Young scientists have become new experts in the field. This increases the potential for healthcare innovations by European academic institutions, hospitals, and industries, backed by the patents on several key findings. Our concepts have been successfully tested for the targeted delivery of the anti-cancer drug doxorubicin and RNA-based compounds against cardiovascular diseases. Extrapolating these results, the platform can be potentially be used for many different drugs, providing opportunities for European pharmaceutical companies. The newly developed technology can further add to the competitiveness of the European industry in the medical field, also due to increasing options for a combination of pharmaceutical and imaging research expanding the portfolio of both the imaging and the pharmaceutical industry. The project members have shared the generated knowledge with scientists and clinicians via publications and presentations. Within SONODRUGS, three courses have been (co-)organized during which we educated SONODRUGS researchers but also scientists from outside SONODRUGS. The concept of US-mediated drug delivery and our results were presented at a dedicated SONODRUGS session of the ESCDD conference. All of these activities may increase the competitiveness of Europe in the medical technology field.

List of Websites:

http://www.sonodrugs.eu