NANOTHER

Integration of novel nanoparticle based technology for therapeutics and diagnosis of different types of cancer

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16	NUOVOPROBE Ltd	NuovoProbe	United Kingdom
17	Argus Chemicals	Argus	Italy
18	ALMA Consulting Group	ALMA	France

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1.1. Project Title

Integration of novel nanoparticle based technology for therapeutics and diagnosis of different types of cancer

1.2. Acronym

NANOTHER

1.3. Start and End Dates

Start Date	1st September 2008
End Date	31st August 2012
Duration	48 months

1.4. Size

€11,540,075.07 Total Budget
€8,408,482.30 EC Contribution

2. Abstract

The breakthrough objective of this project is to develop and characterise a novel nanoparticle system that will be used as a therapeutic agent or diagnosis tool for breast cancer, colorectal cancer and bone metastasis. Theranostics, the development of nanoparticles with both functionalities, will also be carried out using hyperthermic effect to kill tumour cells or to release the selected drug.

Industry and SMEs are a key part in NANOTHER development. The project is based on previously prepared and well known nanoparticles so basic research in this area is limited, but production at high scales is a must. Also, the development of targeted and drug loaded nanoparticles need the scale up process to be carried out, so the result obtained can be used later on in nanopharmaceutical production for therapy and/or diagnosis. Taking all of this into account, industry for high scale production, pharmaceutical companies and nanoparticle developing companies are really interested and involved in project outcomes, patenting and exploitation of the results

The project is divided into seven subprojects from nanoparticle synthesis, antibody attachment, drug attachment and efficacy and biodistribution of the nanosystem. One of the main concerns when producing drugs or therapeutic agents is the toxicity these products can generate in the human body. A specific subproject is dedicated to evaluate the toxicity of the nanoparticles produced which lasts for the whole project and a streamlined protocol for toxicity testing is to be developed. Nowadays, there are no standards for toxicology testing in nanoparticles and in this NANOTHER project; this is a clear objective, although it is not the main one.

Overall the project intents to obtain nanocarriers with stealth capabilities, antibodies in the surface that recognise cancer cell specific markers, drug loaded and the ability of release these drug in a controlled manner.

3. The NANOTHER Project

3.1. Introduction, Scientific / Industry needs & Problem Addressed

Over the last few decades, healthcare costs have been increasing due to a number of factors such as improved diagnostics, generalised screening, improved treatments and the general aging of the European population, which puts ever increasing pressures on healthcare budgets. There is significant pressure on the diagnostic & pharmaceutical industries to maintain innovation and product enhancement in the face of relatively restricted market growth. Nanomedicine and theranostics are emerging markets in which developing technologies and capabilities in nanotechnology, and diagnostic & therapeutic sectors are increasingly converging to improve efficiency and competitiveness of the discovery, development and marketing of diagnostics & therapeutics for various diseases such as cancer.

Nanotechnology is currently the focus of intense development in the field of nanomedicine – the application of nanotechnology to achieve breakthroughs in healthcare. Nanometer-sized particles, such as biodegradable micelles, semiconductor quantum dots and iron oxide nanocrystals, have functional or structural properties that are not available from other existing molecular or macroscopic agents. Recent advances will help towards development of multifunctional nanoparticle probes for molecular and cellular imaging, nanoparticle drugs for targeted therapy, and integrated nanocarriers for early cancer detection and screening. These exciting developments are opening opportunities for future personalised oncology in which cancer detection, diagnosis and therapy are tailored to each individual's profile, and also for predictive oncology, in which genetic/molecular information is used to predict tumour development, progression and clinical outcome. However, a significant challenge when working at the interface between biology and engineering is to ascertain the toxicology and biocompatibility profiles of the materials being used.

The Concept: (Fig 1)

Two of the most important advances in nanotechnology rely on the development of novel technologies for new and effective medical treatments and diagnostic methods. Nanodiagnostics aims to identify disease at the earliest stages - at the cellular level, and perform drug delivery using nanoparticles (NPs) which will enable reduced toxicity of therapeutic drugs by delivering them to the disease site, and the consequent activation of the particles when they reach their target. The breakthrough objective of this proposed NANOTHER European Project is to define and integrate nanoparticle based technology for DIAGNOSIS (labelling of cancer cells) of different cancers and THERAPEUTICS (new targeted drug-delivery system). These multifunctional nanocarriers will have the ability to perform as diagnostic tools for tumour detection and as a therapeutic biocarriers. The cancer types which will be targeted in this project are colorectal cancer,

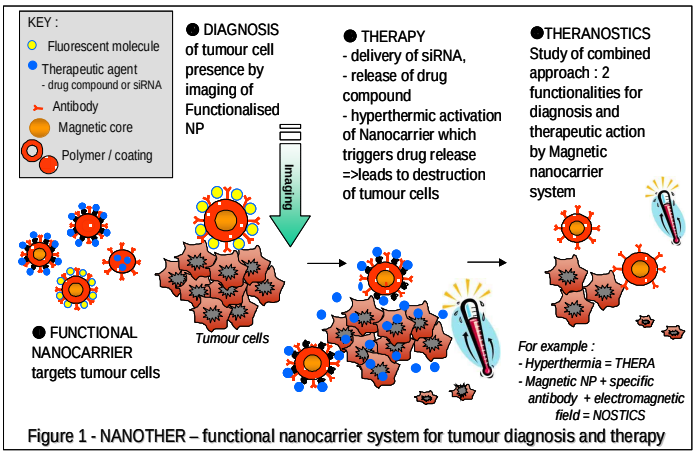


Figure 1 - NANOTHER – functional nanocarrier system for tumour diagnosis and therapy

breast cancer and bone metastasis. Partners of the consortium will also investigate THERANOSTICS by designing nanosized and bioresponsive systems able to give a positive diagnostic and deliver drugs.

NANOTHER aims to select the best nanosystems throughout the project by rigorously testing toxicity & biocompatibility as an integral part of the selection process in order to continue development only on the most efficient, biocompatible and least toxic nanoparticles. An interdisciplinary NANOTHER approach will ensure that the exciting potential of the many facets of nanomedicine will be integrated and improved to become a practical reality in the near future. NANOTHER, at this point, is at the forefront of European technology & industry integration in order to convert current nanotechnology bricks into new, efficient, safe and exploitable nanoproducts.

3.2. Scope & Objectives

NANOTHER intersects biomedical, health and nano industries, and R&D sits at the interface of chemical, biological and physical sciences and engineering. The main NANOTHER objective is therefore based on the integration of 5 key elements of current technology: a) NP functionalisation technology, b) fluorescence / contrast agent & specific antibody diagnostic techniques & imaging equipment, c) novel drug-delivery & activation systems d) new uses for electromagnetic based technology and medical equipment. e) Another important innovation is RNAi technology, and the objective is to investigate the successful formulation and application of nanocarriers including siRNA as the therapeutic agent.

NANOTHER aims to successfully transform nanomaterials into nanointermediates via functionalisation - the linking of antibodies & ligands for detection and binding of tumour cells, and the linking, delivery and release of therapeutic agents to treat the targeted tumour cells. NANOTHER aims to select the best nanocarriers throughout the project by rigorously testing toxicity, biocompatibility, efficacy and biodistribution as an integral part of the selection process in order to continue developing only the most efficient, biocompatible and least toxic nanoparticles. The nanocarriers which succeed in "passing" this rigorous testing process will be selected for continued development and scale-up, and these "successful" nanointermediates can then be considered as future exploitable nanoproducts.

The availability of biopolymers with different behaviour in physiological environments (biodegradability, bioerosion), will be exploited and improved in NANOTHER to adjust the formulation features for better control of drug release kinetics. The co-formulation with magnetically responsive nanoparticles, gives an additional opportunity to control drug release by using stimuli-responsive polymers. These polymers undergo conformational or phase changes in response to variations in temperature and/or pH, thus triggering the release of the drug subsequently to the hyperthermia generated under the effect of EM field.

A major NANOTHER innovation is investigation in to the effect of siRNA delivery via nanoparticles on the 3 types of cancer studied in the project. The pharmacological revolution that RNAi offers is so great that the NANOTHER development effort is an essential and realistic step. Indeed, functional systemic delivery of siRNAs has been reported in a nonhuman primate without any evidence of adverse effects. A major NANOTHER innovation is the inhibition of the hypoxia signalling cascade necessary for tumour growth by silencing the HIF1a subunit using the siRNA approach and the nanoparticles combined with specific targeting ligands to direct the uptake by the target cancer cells as a delivery system.

Another important goal is to explore the advantages of NANOTHER delivery systems with Aplidin, a marine compound, in terms of solubility, administration safety as well as biodistribution modifications in order to improve the rate efficacy / safety of the compound.

NANOTHER will improve methods of image analysis and visualisation, such as 3D optical reconstruction, real-time intracellular tomography, and stereo-imaging. Lastly, NANOTHER

effort will be dedicated to the management of large amounts of data in order to fully gain advantage of imaging results.

3.3. Technical Approach & Work Description

SP1:Tailoring polymer micelle & magnetic core-shell NPs to specific tumour receptors

► Investigation and optimisation of 2 strategies for polymer NP – grafting pre or post NP formation. Inclusion of several antibodies or ligands that bind distinctive targeted cancer antigens: EGFR for colon carcinoma, HER-2 for breast cancer and biphosphonate for bone metastasis, Folic Acid will be employed for recognition of folate receptor over-expressed in cancer cells.

► Characterisation of functionalised NPs (polymer & magnetic) according to different physical methodologies such as photon correlation spectroscopy, NMR, electron microscopy and laser confocal microscopy, light scattering.

SP2:Determination of nanosystem biocompatibility & toxicology

►Assessment of single dose toxicity of nanoparticle carriers selected according to *in vitro* cytotoxicity results. Physicochemical analysis to assess *in vitro* acute toxicity in relation to size & distribution, agglomeration state, crystal structure, production of reactive oxygen species (ROS) using cell lines such as HepG2, 3T3, CaCo2, MDCK, A549, TK6, HaCaT.

SP3:Labelling targeted NPs, visualisation & tailoring imaging systems (MRI, SPECT, PET…)

► Study of diagnosis based on NP possessing a magnetic core, or polymeric NPs labelled with radionucleotides (Tc99) or fluorescent probes (Rhodamine B, FITC). For example, among magnetic NPs, those with appropriate relaxometric behaviour will be chosen e.g. Ferrites associated with MRI (Magnetic resonance imaging). The drug compound Doxorubicin will be used, which is also a fluorescent molecule for imaging purposes.

►Visualisation of targeted & labelled NPs by integration of imaging system & software. Nanocarriers will be studied to preclinical evaluation level by using implantation-relayed assays, growth related bioassays, and angiogenesis related bioassays.

SP4:Formulation of NPs with therapeutic agents - active anticancer molecules & RNAi

►Inclusion of soluble drugs and chemically linked anticancer molecules with selected NPs (organic or hybrid). Kinetic evaluation of drug loading in hybrid NPs.

►Optimisation of drug-loading efficiency, which avoids large drug wastage during particle preparation procedures and reduces the amount of carrier required, with implication also from an economical point of view.

►Formulation of biodegradable NP carriers with siRNA to silence metastasis or tumour associated genes (Fig. 2)

►Design of magnetic drug nanocarriers using Magnetic Fluid Hyperthermia

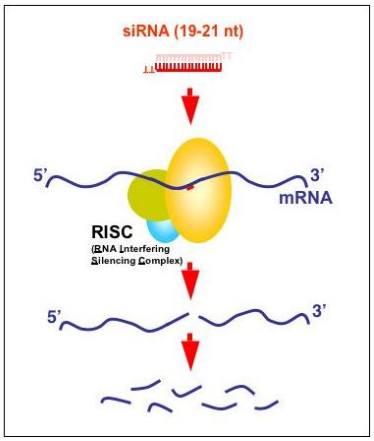


Fig 2: siRNA approach

SP5:Combined theranostic approach

►Design and development of experimental set-up for the contemporary evaluation of combined therapeutic effect and imaging.

► Selection of the best results in therapy and diagnostic to evaluate if they are suitable for a simultaneous application.

►Design of magnetic drug loaded and not drug loaded nanocarriers suitable for Magnetic Fluid Hyperthermia and MRI or PET imaging that heat only the region where magnetic NPs are present and release drugs only to the tumour site, avoiding healthy tissue damage.

SP6: Investigation of NP efficacy & biodistribution in normal and tumour cells *in vitro* and *in vivo* in rats and mice

► Study of *in vitro* efficacy of nanocarriers tested by assessing their cytotoxicity using different cell models representative of the tumour cells of the three types of cancer such as BT-474 for breast cancer, HT-29 for colorectal cancer for instance.

► Study of *in vitro* efficacy of nanocarriers tested by assessing the mRNA levels of the targeted genes using different cell models representative of the tumour cells of the three types like BT-474 for breast cancer, HT-29 for colorectal cancer

► *In vivo* analysis of drug carrier/labelled product efficacy using hepatic metastasis assays, breast metastasis assays and bone metastasis assays after injection of tumour cells in different localisation of the body (intraesplenic, fat pad or left ventricle respectively).

3.4. Conclusions to date& Plans for the Future

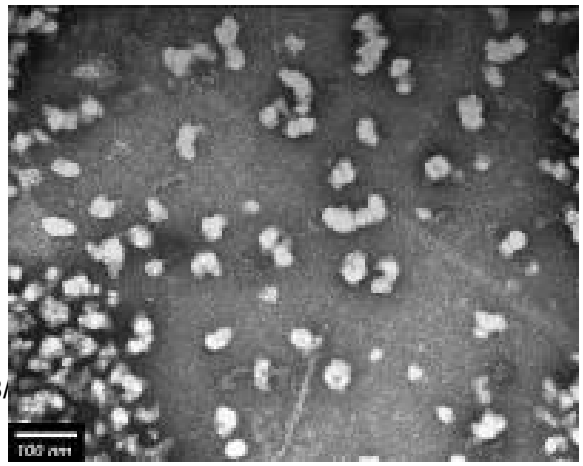
At this point, the project is in M36 out of 48 total months. Several subprojects are already finished like SP1 and SP2. The others are still ongoing. The main results obtained so far are summarized below:

SP1: Tailoring polymer micelle & magnetic core-shell NPs to specific tumour receptors

► More than 10 nanoparticle systems have been produced

► Four polymeric nanoparticles have passed all thresholds regarding scalability, scale up production and manufacturing and look promising for future use. (Fig. 3)

► The conditions to produce these systems are very well controlled and standardised



► More than 5 hybrid systems have been produced
► Two hybrid systems (magnetic core + polymeric shell) have passed all thresholds regarding physico-chemical characterisation.

Fig 3: Some of the nanoparticles produced in NANOTHER

SP2: Determination of nanosystem biocompatibility & toxicology ►
► A streamlined process for nanoparticle testing has been implemented inside the project. It is based on initial screening by *in vitro* basal cytotoxicity methods followed by a more throughout toxicity testing which includes apoptosis/necrosis assays, ROS and inflammation. After this, a full MTD (Maximum Tolerated Dose) and MTMD (Maximum Tolerated Multiple Dose) detailed *in vivo* testing are performed giving a full overview of the toxicity of the systems. Thresholds and concentration ranges have been established according to pharmaceutical companies' standards.

SP3: Labelling targeted NPs, visualisation & tailoring imaging systems (MRI, SPECT, PET…)
► Two polymeric nanocarriers and one hybrid system have been antibody attached and characterised. The antibodies used are at this point commercially available.
► One systems has been loaded with Tc99 for imaging purposes and visualised in mice (see Fig. 4).

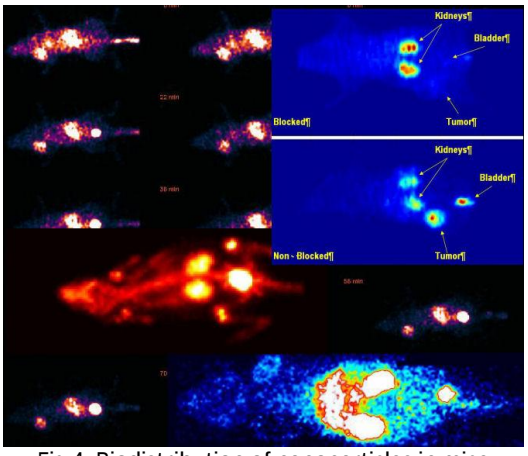
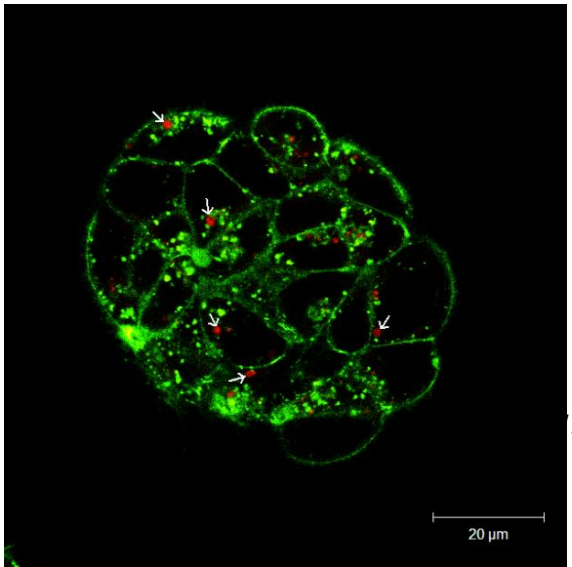


Fig 4: Biodistribution of nanoparticles in mice using Tc99 loaded nanoparticle

► A software tool is under development for 3D modelling and nanoparticle location in 3D.

SP4: Formulation of NPs with therapeutic agents - active anticancer molecules & RNAi
► Nanosystems have been created including several drugs; marine drugs and siRNA containing in some cases more than 20 % w/w of drug/nanosystems-.



SP5: Combined theranostic approach
► Two systems have been developed for theranostics, each of them for use at different electromagnetic field frequencies.

SP6: Investigation of NP efficacy & biodistribution in normal and tumour cells *in vitro* and *in vivo* in rats and mice
► All systems produced and manufactured are ready and synthesized. They are in the

process of testing *in vitro* and *in vivo* for efficacy.
► *In vitro* efficacy has already been performed in systems without antibodies and drugs. Uptake rate and co-localisation studies are under development. Nanosystems seem to internalise well inside the cells tested so far (Fig 5)
►Some assays have already been performed *in vivo* and they look promising with efficacy values similar to those of the drug with the adjuvant used nowadays in cancer treatment but with a much less toxicity

Fig 5: 3T3 cells with fluorescent nanoparticles inside the cytoplasm

The scientific plan for the next and final year involves several aspects. SP1 and SP2 are already finished and no more scientific work will be performed apart from the publications and other dissemination activities planned:

SP3: Labelling targeted NPs, visualisation & tailoring imaging systems (MRI, SPECT, PET…)
► Improving antibody attachment with in-house produced antibodies

SP4: Formulation of NPs with therapeutic agents - active anticancer molecules & RNAi
► Improving drug loading efficacy and capacity if possible for some specific drugs and solving some problems with drug release kinetics

SP5: Combined theranostic approach
► *In vitro* and *in viv*o experimentation is still due and is planned to be performed in the next months.

SP6: Investigation of NP efficacy & biodistribution in normal and tumour cells *in vitro* and *in vivo* in rats and mice
► Uptake rate experimentation has to be finished.
► Experiments with full systems both *in vitro* and *in vivo*

3.5. Copyrights