European Cooperation in the field of Scientific and Technical Research
- COST -

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COST 054/14

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action TD1402: Multifunctional Nanoparticles for Magnetic Hyperthermia and Indirect Radiation Therapy (RADIOMAG)

Delegations will find attached the Memorandum of Understanding for COST Action TD1402 as approved by the COST Committee of Senior Officials (CSO) at its 190th meeting on 14 May 2014.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action TD1402
MULTIFUNCTIONAL NANOPARTICLES FOR MAGNETIC HYPERTHERMIA AND INDIRECT RADIATION THERAPY (RADIOMAG)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4114/13 "COST Action Management" and document COST 4112/13 "Rules for Participation in and Implementation of COST Activities" , or in any new document amending or replacing them, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to address several major issues facing fundamental aspects and clinical translation of magnetic hyperthermia, and to promote it as an anti-cancer treatment in combination with radiotherapy through targeted research, carefully planned cross-disciplinary interaction and dissemination to the general public.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 32 million in 2014 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of section *2. Changes to a COST Action* in the document COST 4114/13.

GENERAL FEATURES

Initial Idea:

In recent years, the emerging field of nanotechnology has paved its way into cancer treatment procedures with the use of nanoparticles for contrast media and therapeutic agents. The combination of conventional cancer therapies with nanotechnologies has shown to be promising in individual clinical studies and bears an enormous potential for the treatment individualisation tailored according to the patient's needs.

This COST Action aims at teaming experienced scientists and young researchers from nanophysics, chemical sciences and medicine for improving the knowledge of combined cancer therapies. Particular attention will be paid to the increase of the radiotherapy efficiency and its combination with magnetic hyperthermia. These new findings, obtained under the coordination framework of this Action, will results in a better dose optimisation confining cell damage to tumour regions only, under concurrent exploitation of sophisticated radio-surgical tools already available in hospitals. Furthermore, proper dissemination of scientific results to the broad public and possible stakeholders is another important concern of this Action.

The improved knowledge resulting from the proposed coordinated, target-oriented interdisciplinary exchange will encourage industrial partners to produce a new generation of magnetic nanoparticles suitable for diagnosis, chemotherapy, radiotherapy and magnetic hyperthermia. Promoting the application of combined cancer treatments will contribute to a better individualised treatment planning for cost-efficient cancer therapies covered by state health insurances.

Keywords: combined cancer treatment, magnetic hyperthermia, radiosurgery, coating of bio-magnetic nanoparticles, magnetic and bio-relevant property characterisation.

A. CHALLENGE

The death rates attributed to cancer are amongst the highest in the European community. In 2010, about 70 per 100 000 inhabitants aged younger than 65 died from cancer, which is almost twice than the death rate for circulatory diseases according to EUROSTAT. In Europe, lung, colorectal, breast

and stomach cancer are the most frequent and account for half of all deaths. Even if not the most frequent, one form of brain cancer called glioma, is one of the most dramatic, especially if a relapse occurred after a primary treatment, with a median patient survival of 9 to 12 months. Such a poor prognosis is actually a clear indicator of the resistance of tumour cells against common therapeutic strategies, especially external radiotherapy (ERT). Similarly, despite the development of targeted therapies and immunotherapy (anti CTLA4), high grade melanoma, an increasingly frequent skin cancer with distant metastasis, have a poor outcome and prognosis. A major challenge is to combine different strategies in order to maximise tumour cell damage by inhibiting deoxyribonucleic acid (DNA) repair through chemotherapy and/or ERT, decreasing the risk of tumour relapse.

In view of these facts, research on cellular processes initiating cancer formation and the immune response to tumour genesis is much needed in order to develop new medicines and treatment strategies and to ameliorate cancer prophylaxis. New approaches are necessary at the same time for improving existing treatment techniques in order to minimise deleterious side effects and to increase the patient survival rates. There are many opportunities for new and efficient drug delivery systems, in particular for nanovectors carrying a therapeutics combined with imaging probes. Surgery, chemotherapy, and radiotherapy are the most common conventional therapies currently applied. Their combination with nanotechnological approaches has shown to be promising in individual clinical studies and bears an enormous potential for personalised medicine. This COST Action lies within the context of the described framework.

The therapeutic approach whereby elevated temperatures damage and/or kill malignant cancer cells within the body is known as hyperthermia. Although the positive effect of heat for tumour treatment is known for long time, hyperthermia has not evolved noticeably due to the lack of appropriate heating methods able to localise the temperature increase in tumour tissue. For three decades, the use of electromagnetic radiation for cancer treatment has been widely discussed in the scientific literature. In vitro studies on cell cultures have shown that hyperthermia provokes programmed cell death (apoptosis) as well as premature cell death (necrosis), depending on cell type and on the temperature applied.

During the last decades, hyperthermia got a boost with the emerging nanotechnology, incorporating the use of coated magnetic nanoparticles as local heat sources. This method is known as magnetic hyperthermia (MH). When exposed to alternating magnetic fields of appropriate intensity and frequency, these particles release heat due to re-magnetisation processes. The advantage over

conventional heating methods is that heat is only locally induced, i.e. where magnetic nanoparticles are concentrated.

The synergetic effect of ionising radiation and hyperthermia in killing cancer cells is well-known since cancer cells resistant to radiation but sensitive to hyperthermic conditions were discovered. Notwithstanding, thermo- and irradiation sensitivity of cancer cell types depends on the stage of cancer growth. Combining both therapy concepts, one expects an increased efficiency of the radiation treatment by prior application of moderate MH and to possibly decrease the delivered radiation dose or to enhance the radiation effect on cellular hypoxia by increasing the local energy deposition related to the interaction between the radiation beam and magnetic nanoparticles. The radiosensitiser effect of nanoparticles related to the enhancement of the photon absorption proportional to the cubic electron density (Z) was previously proposed for gold and gadolinium oxide. Despite the lower Z value of the iron and iron oxide, it is argued that this effect will also contribute to enhance the cell damaging power of the ERT in tumour cells and finally improve the survival rate of cancer patients with poor prognosis as glioma or melanoma.

In this context, fractionated stereotactic radiotherapy (CyberKnife, Gamma knife) is of particular interest because it is a modern established anti-cancer therapy applied in many hospitals, and innovations in this field are being supported by e.g. COST Action TD1205.

Because of their small size, nanoparticles are easily transported around the human body and may penetrate physical and physiological barriers. For instance, the blood-brain-barrier restricts access to the cerebrospinal fluid for most chemotherapeutic agents but certain kinds of nanoparticles are able to cross it, permitting entrance to the central nervous system (CNS). This can be used to target CNS tumours which are difficult to reach otherwise. In order to provide these nanoparticles with a direct radiosensitising effect beyond their mere composition, a clear cell membrane crossing is crucial to maximise the dose deposition close to the main target of the ERT, i.e. the DNA.

Currently, MH in humans is efficient only when used through direct injection of the magnetic fluid inside the tumour site. Therefore, the main challenge is to achieve an efficient targeting of tumour cells providing a strong hyperthermia effect using the lowest magnetic nanoparticle concentration possible, becoming an adjuvant therapy for all tumour sites, primary and metastasis via parenteral, for instance intravenous, injection.

The general idea of the proposed COST Action is to address some major issues of magnetic hyperthermia and to promote and to leverage it as anti-cancer treatment in combination with radiotherapy.

The strategy to achieve this goal consists of following action objectives:

- **Coordination of experimentation or testing (category A3),**
- **The development of knowledge needing international coordination (category A5),**
- **Dissemination of research results to the general public (category A9),**
- **Bridging separate fields of science/disciplines to achieve breakthroughs that require an interdisciplinary approach (category B13).**

Action Objective: A3 (Coordination of experimentation or testing) & B13 (Bridging separate fields of science/disciplines to achieve breakthroughs that require an interdisciplinary approach)

Standardisation and coordination of magnetic hyperthermia experiments

The heating efficiency of magnetic nanoparticles is assessed by a physical quantity called specific loss power (SLP), also known as specific absorption rate (SAR). It represents the thermal energy per unit mass and time, released by magnetic nanoparticles during remagnetisation processes in alternating magnetic fields. Although the use of the SAR parameter has become widespread in the hyperthermia community to evaluate the heating capabilities of magnetic nanoparticles and to establish exposure limits, the outcomes from different experiments cannot be easily compared. Consequently, a new parameter, the so-called intrinsic loss parameter (ILP) has been proposed as a first approximation to allow a more direct comparison between results from measurements carried out in different laboratories under different field strengths and frequencies.

Strictly speaking, the ILP parameter is a low-field approximation to a more complex relationship, and is best used subject to the following conditions: (i) field frequencies of up to several hundreds of MHz; (ii) polycrystalline samples with a polydispersity index of less than 0.1; (iii) applied field magnitudes well below the saturation field of the nanoparticles; and (iv) experimental conditions wherein environmental thermodynamic losses do not overcome the power input from the

hyperthermic field. Nevertheless, even with these provisos, the ILP parameter is a useful comparator in any discussion of particle performance.

The research community would benefit from making more efforts to publish comparable data from experimental studies and clinical trials under appropriate conditions by:

- adopting standardised parameters such as the cumulative equivalent minutes at 43 ºC (CEM43), which is the accepted metric for thermal dose assessment,
- using the ILP parameter instead of, or alongside, the SAR parameter, enabling better comparisons with regard to the magnetic heating properties of the given particles,
- revising the Brezovich criterion in MH in the view of the most common or desired experimental conditions for different types of cancer, carrying out dedicated experimentation to assess its usefulness. This criterion, which establishes a top value for the field frequency/amplitude product, is being used in many publications as the safety "golden rule" for clinical MH, even though the experimental conditions upon which it is founded are not comparable with those employed by researchers nowadays.

Action Objective: A5 (Development of knowledge needing international coordination: improved model / technology) & B13 (Bridging separate fields of disciplines to achieve breakthroughs that acquire an interdisciplinary approach)

This objective concerns about stimulating the interdisciplinary exchange of scientific research through coordinated, target-oriented research. In particular, the Action will shed light on the following aspects: methods and enrichment processes of magnetic nanoparticles in tumour tissues, optimal magnetic properties of coated nanoparticles, coating/targeting of magnetic nanoparticles. All these different research topics are interdisciplinary, and the research community would benefit from the strong exchange of knowledge and know-how.

Controlled enrichment of magnetic nanoparticles in tumour tissue

One of the approaches for delivering therapeutic agents to tumours is passive targeting (enhanced permeability and retention "EPR" effect). It takes advantage of both, the high permeability associated to the tumour vasculature and the fluid retention caused by its defective lymphatic system, entailing particle accumulation over time in the affected tissue. However, the optimal approach is active targeting, which makes use of either locally or systemically administered nanoparticles functionalised

with specific ligands recognised by tumour cells that specifically bind to the targeted tumour, constituting a first step towards tailored treatments.

Although much progress has been made in this area, it is still difficult to achieve targeting in a clinical setting. There has been a burgeoning activity around active targeting during the past ten years, but it has been lately taken to a higher level with the design of multifunctional nanocarriers. The potential of both active and passive targeting needs to be investigated and discussed in dependence of the tumour type.

Theoretical and technical challenges in magnetic hyperthermia

The major pitfall of magnetic hyperthermia is an imprecise experimental determination of the temperature field during the treatment, leading to either insufficient heating or, on the contrary, over-heating and collateral damage of healthy tissues. Thus, temperature should be measured both spatially and temporally, for a proper determination of the thermal dose CEM43, defined as the time integral of the temperature curve above 43°C, inside the tumour and in its periphery. Usually temperature is measured using fibre optic probes, whose captors are insensitive to radiofrequency magnetic fields. However, those probes have a diameter of 200 µm at least and cannot be precisely implanted in a tumour. Among the different possibilities such as ultrasound echography, optical near-infrared and magnetic resonance imaging (MRI), MRI should be firstly envisaged, since it can offer several thermometry methods: measurement of the nuclear spin- lattice relaxation time (T1), diffusion MRI, and proton resonance frequency. The latter method is the most sensitive one, showing a ±1.0°C precision. The main challenge consists in isolating the very sensitive detection antenna electronics from the strong magnetic field of the radiofrequency hyperthermia inductor.

In order to optimise a magnetic hyperthermia treatment in vivo, it is necessary to predict the expected temperature distribution in and around the tumour as a function of the intensity and the application time of the external magnetic field, which is done by mathematical modelling (bio-heat transfer equation). Currently, macroscopic heat transfer analysis is used to assess the energy distribution in and around a tumour. This theoretical concept is not the best for treatment planning, because there seems to be no advantage to deliver heat using nanoparticles. Instead, recent experimental data have shown that nanoscale thermal effects exist due to energy dissipation when nanoparticles are exposed to alternating fields. At the nanoscale, the existing macroscopic heat transfer analysis is no more appropriate, which explains contradictions between experimental data and modelling according to investigations of a few individual research groups.

The combination of MH with radiotherapy would definitely benefit from a common concept of both, thermal and gamma-ray energy. Future patients would benefit from better dose planning and lower side effect of the treatment.

Design of magnetic nanoparticles and their structural and magnetic characterisations

One of the limitations of MH is the low heating power of the usual magnetic nanoparticles used in in vivo tests, requiring a local injection of large quantities of nanoparticles. Therefore, the challenge is to maximise the heating power of the existing magnetic nanoparticles. The amount of heat generated depends strongly on nanoparticle structure and magnetic properties. Most studies have been performed on spherically shaped nanoparticles, and an optimal size of 20 nm has been determined. Current progress in the synthesis of iron oxide nanoparticles with different shapes and compositions (including core-shell structures) show that the heating power can be optimised. For example, the combination of two materials with different magnetic anisotropy within the same nanoparticle, e.g. a core with high magnetic anisotropy and a shell with low anisotropy, significantly improves the heating power of nanoparticles through an exchange mechanism. Another strategy for increasing the heating power induced by the particles is an appropriate architecture (cubic or flower nanostructures) that also strongly affects the magnetocrystalline anisotropy. Magnetic nanoparticles have recently shown considerably improved properties for hyperthermia with SAR values above 1000 W/g, even for pure iron oxide.

The heat released by a system of magnetic nanoparticles not only depends on their intrinsic properties, but also on the interaction between individual particles. The latter is an important issue intimately related to the efficiency of magnetic hyperthermia agents that has not been properly addressed in the past years. In a fluid containing superparamagnetic nanoparticles (i.e. no magnetic hysteresis), the collective behaviour is different from that of isolated particles when a magnetic field is applied. For larger particles, near the superparamagnetic / stable single domain size threshold, dipolar interactions between nanoparticles are even stronger and play a role in the relaxation process.

The degree of dipolar interactions depends also on the coating thickness that separates the individual particles. Better knowledge about magnetic particle interactions in living tissue is of importance for a better understanding and determination of the ILP in vivo and for modelling the transfer of heat inside tumours.

From a magnetic point of view, a delicate balance has to be achieved between the intrinsic properties deriving from the material structure (e.g. anisotropy field, saturation magnetisation) and the experimental conditions to be employed, such as frequency and amplitude of the applied magnetic field.

Coating of magnetic nanoparticles ensuring biocompatibility, controlled bio-distribution and targeting

In general, crystalline magnetic particles are foreign matter for the human body, and are eliminated by the mononuclear phagocyte system, which is a part of the immune system. Consequently, they are coated with organic molecules to enhance their biocompatibility, bio-distribution and escape from the reticuloendothelial system (RES). In this sense, the challenge is the development of an appropriate organic coating design, because the latter is a key point for biomedical applications such as dyes for optical imaging, targeting ligands to reach target tissue or cells, and therapeutic agents (drug delivery) allowing a rapid elimination of particles that have not reached their target. In order to prevent agglomeration in a physiological environment and opsonisation of nanoparticles, the organic coating and its anchoring at the nanoparticle surface need to be tailored by increasing their ability of bypassing the RES and favouring their bio-distribution and -elimination.

The final average hydrodynamic sizes of the nanoparticles have to be in the range 10–100 nm, favouring long-term blood circulation for successful in vivo delivery. Therefore, molecules and grafting strategies have to be designed to fulfil all these requirements. The following ones are considered as reasonable candidates:

a) Polymer coating

Hydrophilic biocompatible polymers such as dextran facilitate dispersion in aqueous media and confer to the nanoparticles a chemical stability in blood plasma. Therefore magnetic nanocomposites obtained by coating magnetic nanoparticles with polymers represent good candidates for biomedical applications. Their surface, rich in functional groups, represents an advantage in drug delivery, such as an ability to cross biological barriers, drug protection from rapid degradation in biological systems, and provision of a large surface area for conjugating targeting ligands.

Magnetic nanocomposites with controlled properties are prepared based on a dense packing of superparamagnetic nanoparticles, well separated by the adsorbed organic stabilising layer preserving their superparamagnetic behaviour.

Surface coatings with attached functional groups, like carboxylic or amino, and click chemistry groups can be prepared based on several chemistry routes, allowing the attachment of specific biomolecules that improve the interpenetration through biological barriers.

The responsive properties of polymer coatings in response to external stimuli like temperature represent a new promising route to improve drug formulations for existing therapies with reduced side effects. Magnetically responsive nanocomposites, like nanogels, micelles or vesicles (liposomes or polymersomes) exhibiting a critical polymer solution temperature, offer new possibilities of triggered drug delivery at the tumour site. The heat locally generated by the magnetic nanoparticles in alternating magnetic fields is transferred to the thermo-responsive organic coating, inducing an enhanced release of drugs initially trapped. Such innovative combination of magnetically guided targeting, thermotherapy and chemotherapy is at the forefront of current research in nanomedicine.

b) Dendron coating

Polymer coatings may lead to a large average hydrodynamic size of nanoparticles in suspension and heterogeneous size distribution of coated particles. Besides polymers, another class of molecule is emerging, small dendrons. Indeed, the use of dendrimers or dendron discrete building blocks for biomedical applications is a flourishing area of research, mainly due to their precisely defined structure and composition, and highly tuneable surface chemistry. Dendrimers and dendrons are promising due to the diversity of functionalisation brought by the arborescent structure that simultaneously solves the problems of biocompatibility, low toxicity, and large in vivo stability. They allow a versatility of size and properties precisely tuned as a function of their generation. The use of such small dendrons appears as a good way to ensure, after the grafting step, a mean particle size below 50 nm together with a narrow size distribution in suspension, both being prerequisites for a good biodistribution, i.e. avoiding RES uptake and rapid elimination by renal and/or hepato- biliary ways. Grafting of dendritic molecules onto the surface of nanoparticles using a phosphonate group as coupling agent has led to a new generation of contrast agents for MRI.

c) Targeting properties

The molecules to be grafted onto the surface of nanoparticles have to provide a small overall particle size and ensure their multi-functionalisation (e.g. through dyes, targeting ligands). To contribute to a better understanding of the therapeutic effect at cell and tumour levels, besides toxicity measurements, the internalisation of these nano-objects in cells or tumours either via usual

endocytosis (e.g. clathrin-mediated) or by active targeting (by grafting targeting functions) needs to be investigated to determine the cellular and/or tumour internalisation pathways.

Action Objective: A9 (Dissemination of results to the general public)

The potential of magnetic hyperthermia has been demonstrated recently in a clinical phase II study, with patients suffering from recurrent malignant brain tumours. The treatment with magnetic hyperthermia and adjunct fractionated stereotactic radiotherapy resulted in slightly increased survival times compared to current chemotherapeutic standard treatments for such type of cancer. In order to estimate the potential of the magnetic hyperthermia treatment as anti-cancer therapy, which would potentially increase the patient survival time, randomised and multi-arm clinical trials are highly desirable. However, recent Phase II clinical trials of magnetic hyperthermia in combination with radiotherapy are not yet in accordance with these demands. Randomness is an important element in clinical trials in order to avoid possible biases due to unknown, patient related factors. Achieving randomisation in such a clinical trial is ethically complicated because selected patients would benefit from the experimental treatment while those from the control group would not. In addition, broad and proper dissemination of scientific knowledge is necessary to reduce prejudices and barriers, to promote understanding for clinical trials and to improve the impoverished perception on medical advances.

B. ADDED VALUE OF NETWORKING

The overall aim of this Action is to promote and to leverage magnetic hyperthermia (MH) for anti-cancer treatment in combination with radiotherapy. The transition from the experimental stage towards a widely accepted treatment requires common experimental standards and testing procedures, as well as systematic studies of coating and magnetic properties of the active agents in order to propose feasible treatment strategies for clinicians. These are not currently in place and clinical trials are rare, albeit the potential of magnetic hyperthermia has been demonstrated in experiments based on a local direct injection of magnetic nanoparticles. Current international conferences deal only partly with the advances in the field of magnetic hyperthermia. Although there are efforts to stimulate interdisciplinary exchange and to actively animate discussion between scientists from different research fields, no concrete strategic plans about the coordination of research and the future development of magnetic hyperthermia have been set up yet. There is neither common research centre

nor network dealing with research in magnetic hyperthermia and its combination with radiotherapy. In contrast, electromagnetic wave hyperthermia is closely incorporated into clinical research and considered as part of standard anti-cancer treatment.

The so-called theranostic nanomedicine – an integrated therapeutic system that can diagnose, deliver a therapy and monitor the individual's response to it – is strongly supported by the European Commission; at least three FP7 projects were dealing with such strategy. These projects were mainly concerned with research and development activities primarily focused on a polyvalent approach to an anti-cancer treatment, including market studies. In contrast, the proposed COST Action aims to bring together and to organise the research outcomes from the different participating network members in a practical way to provide clinicians with the necessary input to trial a novel anti-cancer treatment combining magnetic hyperthermia and radiotherapy, also identifying future research objectives upon appraisal of the obtained results. Feedback between the different Working Groups here is essential, and is expected that the lifetime of this COST Action will eventually result in a compendium of best practices for magnetic hyperthermia.

The integration level provided by a pan-European network is of paramount importance for the advancement of magnetic hyperthermia as a niche research. Noteworthy, it will allow for:

- a deeper insight into the current situation on each participant country about the actual degree of spread of any related technique to the magnetic hyperthermia, either as a standalone or adjunct therapy for cancer. This will help in further shaping the initial milestones set for this Action and also ease the design of a sensible bench-to- bedside roadmap;
- a more efficient exploitation of human and instrumental research resources available, shortening experimentation times and making possible the realisation of complex/coordinated experiments otherwise impossible to carry out;
- avoiding duplicities in research efforts through a fluent communication and data exchange; Screening processes to speed up and identify any pitfalls in existing protocols will become straightforward;
- enhancing the congruence between technical development and clinical challenges, between pre-clinical assessing and medical input, by associating clinicians and physicians in a strong multidisciplinary network.

Action Objective A3 (Coordination of experimentation or testing)

This Action Objective will bring together theoretical and experimental scientists in order to work out a common standard protocol for determining the energy absorbed by biological tissues, being supported by a new theoretical concept taking into account nanoscale thermal effects. This Action Objective falls into category A, because it requires intensive exchange and collaboration between participants as to put forward a common, clear and well-grounded standard procedure for optimising the energy loss parameter (see section A, objective A3) in order to be easily employed by clinicians at a later stage. Such standardisation cannot be achieved without international coordination (category A), because:

- experimental equipment and measurement procedures are different in individual universities and research centres across the world;
- an agreement about standard procedures for experimental validation of the heat distribution in tumours does not exist, but is required for the advancement of magnetic hyperthermia as anti-cancer treatment, by taking account the strong dependence to the type of tumour, the location of the xenograft, and the gaps between small animal tumour model and the human cancers to accelerate the clinical transfer;
- of a different perception of the concept of electromagnetic energy absorption by living organisms used in magnetic hyperthermia by individual research groups.

The last point is an important issue, since different names are circulating in the scientific literature for the amount of thermal energy generated by remagnetisation processes. For example, the term specific absorption rate (SAR) is appropriate when considering a medium exposed to electromagnetic radiation, while the term specific loss power (SLP) is rather convenient for mathematical determinations. An appropriate use of both terms, although used synonymously in the literature, is important, as their determination relies on different physical principles. This will also remove ambiguity, resulting in a more straightforward interdisciplinary scientific exchange with medical scientists. On the one hand, the SLP represents a theoretical value calculated on the basis of a physical model, involving also the measurement of magnetic parameters such as the imaginary part of the magnetic susceptibility. On the other hand, the SAR is determined by temperature vs. time measurements in physical experiments. Although the SAR is determined in this way, it does not necessarily correlate with those of nanoparticles in biological tissues. In the latter, cell membranes and their geometrical arrangement constrict the heat transport, which is not the case in a truly physical

environment, where a ferrofluid is able to circulate freely. A consensus is necessary in order to translate magnetic hyperthermia into an effective cancer treatment. Standardisation of hyperthermia experiments incorporating also biological parameters as proposed will result in a more accurate treatment planning for magnetic hyperthermia.

Action Objective A5 (Development of knowledge needing international coordination: improved model / technology)

Action Objective 5 concerns mainly about those aspects of science and technology requiring a close collaboration between scientists of different disciplines. This COST Action will serve as a platform for scientific discussion and exchange of ideas in the area, which is eagerly demanded. For instance, current progress in nanoparticle synthesis allows the creation of several magnetic particles with different composition and shapes, holding sufficient promise for enhancing the hyperthermic effect. In order to better understand the role of particle shape, interaction and composition on the hyperthermic effect, and hence to select by targeted approach the most suitable one, discussion and exchange is indispensable. Moreover, this holds true when compromises have to be found between the feasibility of the nanoparticle fabrication process, the heating efficiency, coating / targeting properties and economic/environmental framework conditions. Another example underlining the demand for discussions and exchange, concerns the upper limits of magnetic field intensity and frequency range to be consistent with the EU directive (2013/35/EU) on the minimum health and safety requirements regarding the risks upon exposure of workers to electromagnetic fields. Current pre-clinical experiments use for instance a ten times higher field intensity × frequency product than that suggested by the EU directive.
There is neither a common agreement on safety limits of magnetic hyperthermia nor it is clear which way is best to quantify MH induced cytotoxicity.

The RADIOMAG Action will generate new and strengthen the existing synergies between technical advances (thermal imaging / MH), new treatment concepts (combined targeting radiosensitisation and magnetic thermotherapy) and biocompatible coating in order to achieve a breakthrough in the clinical application of magnetic hyperthermia. Due to the complexity of this aim, synergies can only be achieved on a longer time frame, by means of workshops, STSMs, Joint Peer-Reviewed Publications, common Horizon 2020 research proposals and exchange with other COST actions (e.g. TD1004, TD1205). Several top-class research groups have agreed to join this Action. Their fruitful exchange of scientific knowledge and know-how under the patronage of COST is necessary to be at

the forefront of MH research, particularly in view of the actual trends showing an outward migration of talents from Europe and increasing research efforts in Asian countries.

Action objective A9 (Dissemination of results to the general public)

Reach out to the public is another concern of this network, but it will be done keeping in mind the somewhat deteriorated public's view on medical innovation in the past years. The intense media interest raised by some breakthroughs in biomedicine, such as the development of new drugs, frequently leads to unrealistic expectations by both, patients and the broad public, mostly seized by the hope of immediate therapeutic results. This situation, if possible, further deteriorates when the risks associated with new therapies are not properly communicated to the wide audience in an appropriate context. The aim is therefore to access the existing public engagement mechanisms at the consortium's reach, ensuring a responsible and more realistic communication of results, improving the impoverished perception on medical advances. At the same time, this COST Action will prioritise an accurate representation of the environmental impact of using nanoparticles in healthcare. In order to reach out general public, patients and possible stakeholders, the scientific results of this Action will be edited in a generally understandable manner. This requires collection of information from the participating parties, discussions between the different interdisciplinary partners, the provision of means / platforms channelling the information to be distributed for proper dissemination. COST networking tools such as Working Group meetings and Action Conferences will allow a successful achievement of this objective, particularly because of its interdisciplinary character. In contrast to two other comparable running COST actions (TD1004, TD1205), this Action will have a specific Working Group dedicated to public relations. This objective cannot be realised without international coordination (category A), because the production of well-articulated dissemination material requires consensus among the different scientific disciplines in order to speak a common language. Already during the proposal writing process it became evident that partners from different disciplines have a different perception on specific terms and definitions. The scientific exchange between international partners is therefore essential for achieving this objective since, a fortiori, possible stakeholders are also active at international level.

Action Objective B13 (Bridging separate fields of disciplines to achieve breakthroughs that acquire an interdisciplinary approach)

The Actions Objectives proposed in A5 are of strongly interdisciplinary character and require mutual interaction between the different disciplines represented by the participating researchers. Expertise in physics is required for testing magnetic and heating properties of magnetic nanoparticles, knowledge and experience in chemistry are necessary for coating / fabrication, whereas bio-molecular technologies are involved in targeting and fabrication of magnetic nanoparticles. Finally, a solid medical competence – especially in oncology and radiology – is indispensable for translating the former concepts into clinical trials of magnetic hyperthermia. However, keeping an up-to-date overview about methods, publications and the newest technologies in all those research fields involved in Action Objective A5 is difficult for individual research groups in established academic structures. This is exemplified by the relentless growth in related scientific publications; for instance, about 5000 new articles and over 350 patents dealing with magnetic nanoparticles were published in 2012 alone, according to Scopus and the European Patent Office, respectively.

This Action Objective, i.e. bridging separate fields of sciences, can only be achieved in a longer time frame (category B) without a COST action because:

- coordinated research and exchange targeting on magnetic hyperthermia do not exist yet in Europe;
- researchers belong to different universities and research centres with different scientific priorities and hence they do not focus exclusively on MH;
- the amount of new scientific information is enormous and has to be filtered in order to efficiently achieve the goals of this Action Objective.

In view of the steady progress in science and technology, stimulating and promoting lateral thinking and interdisciplinary working becomes more and more important for future researchers. This is particularly relevant for early stage researchers who finished their studies after the introduction of the Bologna process. The latter does not appear to be implemented throughout European countries as equally as it was expected in principle, and has resulted partly in a constriction of the teaching contents, limiting the desired interdisciplinary coverage. Being part of the structural and functional organisation of the RADIOMAG network will benefit any of these early stage researchers eventually involved in this COST Action.

C. MILESTONES AND DELIVERABLES: CONTENTS AND TIME FRAMES

STRATEGY

Objective 1 (A.3) - Type: Coordination of experimentation or testing

1. Action Science and Technology Meeting, Working Group.
2. Handbook, Guidelines, Best Practices, for S&T purposes.
3. Unpublished Aspects of Knowledge Creation, Including Experimentation and Testing, scientific experiment or test.
4. Science and Technology Coordination, Short-Term Scientific Missions (STSM).

Objective 2 (A.5) - Type: Development of knowledge needing international coordination: improved model / technology

1. Joint peer-reviewed publication, open access.
2. Action Science and Technology Meeting, Working Group.
3. Science and Technology Coordination, Joint Student Supervision (at Master's or Doctoral Level).
4. Science and Technology Event or Meeting, Action Conference.
5. Science and Technology Coordination, Short-Term Scientific Missions (STSM).

Objective 3 (A.9) - Type: Dissemination of research results to the general public

1. Internal and External Communication, Website.
2. Internal and External Communication, Production of dissemination material for distribution.
3. Action Science and Technology Meeting, Working Group.
4. Delivery of Written Input to a Stakeholder (excluding business enterprises), to users/practitioners.

Objective 4 (B.13) - Type: Bridging separate fields of science/disciplines to achieve breakthroughs that require an interdisciplinary approach

1. Achievement of Specific Network Features in terms of WG Composition, expertise.
2. Science and Technology Event or Meeting, Action Workshop.
3. Science and Technology Event or Meeting, Training School.
4. Contacts with Stakeholders, Input for the Formulation of Framework Programme Calls.
5. Action Science and Technology Meeting, Working Group.

According to the problems explained and described in “Challenge” and objectives given in the “Strategy” the following milestones and deliverables are proposed here as solutions.

Objective A3 – Coordination of experimentation or testing

***Milestone A3-1:*Standard procedure for estimating the thermal dose (e.g. CEM43, ILP), safety standards, and the investigation of particle tissue interaction.**

This milestone concerns the establishment of a protocol for calibration of experimental set-ups, the selection of a standard calibration ferrofluid as well as a standard measurement procedure for the determination of the intrinsic loss parameter (ILP). These measures will allow a facile comparison of ILP results from different laboratories. This is necessary for further research tasks to be carried out in the individual laboratories of the network members, such as systematic ILP determination of different ferrofluids taking into account the ratio between nanoparticle size and coating thickness, including adhered therapeutic / radio-sensitising agents, or to evaluate the influence of grafting on the magnetic properties. The same policy will be applied for in vitro or in vivo experiments involving biological tissue. Also here, no standardised protocol exists for quantifying heat delivered to biological tissue, although temperature measurements are more complex compared to truly physical experimental set-ups. The use of a common protocol is necessary to compare the efficiency of magnetic hyperthermia in pre-clinical and clinical trials.

The suitability of the Brezovich criterion for field intensity and frequency will be revised in the light of the most common or desired experimental conditions for different types of cancer and more experimentation to assess its usefulness will be carried out.

The exact approach will be discussed in a Working Group (WG) meeting shortly after this COST Action commenced. One of the partners will be in charge of further organising and monitoring the standardisation / experimental procedures, which will be carried out in different laboratories of the network. Short-Term Scientific Missions (STSM) during the same year will allow an exchange of practical experience / know-how and to evaluate inter-laboratory calibration results. Final results will be presented in the second year during an Action Science and Technology Meeting, and finally a written document guidelines will be issued, available for network members and third parties.

Types of milestones/deliverables & envisaged time-frame

- Action S&T Meeting: Approach determination (1st year)

- STSM (1st & 2nd year)
- Unpublished Aspects of Knowledge Creation, including Experimentation and Testing: exchange of experience and inter-laboratory calibration (1st & 2nd year)
- Action S&T Meeting: Discussion of final results (2nd year)
- Guidelines: issuing written document available for network partners and others dealing with magnetic hyperthermia research (2nd year)

Objective A5 – Development of knowledge needing international coordination: improved model / technology

Milestone A5-1: **Targeting strategies for tumours.**

This milestone concerns the establishment of Working Groups and discussion platforms with participation of bio-chemists, cell biologists, chemists, physicists and oncologists in order to analyse existing coating methods and to put forward new coating strategies in passive and active targeting. Concretely, this milestone will elicit the technical feasibility and the scientific reasoning of a multimodal coating, that:

- eases the entry of coated magnetic nanoparticles into cancer cells (e.g. outer shell of glucose),
- reinitiates mitochondrial activity (e.g. dichloroacetate, lipoate) and slows down glycolytic energy production (oxamate), in order to release apoptotic signals in cancer cells,
- includes radiation enhancers (e.g. lutetium) to minimise the necessary intensity of the primary radiation during radiotherapy.

Further strategies that will be explored and improved are macrophage-based targeting methods and multifunctional coating. This new knowledge will help to optimise the enrichment of coated magnetic nanoparticles in tumour cells, possibly also without surgical intervention (i.e. active targeting).

Milestone A5-2: **Advancement of theoretical and technical concepts of magnetic hyperthermia.**
As stated in section A, coupling magnetic hyperthermia with precise thermometry inside and around a tumour is envisaged, as well as the evaluation of new and existing heat transfer models for a more efficient application of MH. The models and techniques developed by individual partners will then be tested, discussed and evaluated. This concerns for instance: (i) the energetically efficient design of the radiofrequency coil and generator to create a magnetic field on large volumes (for in vivo

experiments) with well characterised and controlled field homogeneity, (ii) the real time modulation and modelling of the applied magnetic power to deposit precise thermal doses, or (iii) the establishment of an alternative theory of nanoscale energy transfer based on the concept of phonons that will be tested by experimentally determined intrinsic loss parameters.

Milestone A5-3: **Improved design of magnetic nanoparticle / structural and magnetic characterisations for better MH efficiency.**

The research to be carried out concerns systematic coordinated studies on the synthesis of oxide and metallic nanoparticles displaying different shapes and compositions (including core-shell structures and clusters) proposing a reasonable strategy for increasing the heating power of the nanoparticles. Also, coordinated experiments will be carried out investigating a possible significant influence of coating and clustering properties on the specific absorption rate and the ILP, theoretical calculations of secondary radiation taking into account the coating properties. The nanoparticles will be characterised structurally and magnetically. In particular, the anisotropy induced by the shape and/or the core-shell and cluster structure will be determined and correlated to their MRI and hyperthermia properties.

Milestone A5-4: **Improved coating of magnetic nanoparticles ensuring biocompatibility, biodistribution and targeting.**

This milestone will be achieved via discussions and joint experiments concerning the selection of multifunctional coatings providing good biocompatibility, bio-distribution and targeting. Multifunctional polymer and dendron coatings will be envisaged. In particular, further research efforts will be devoted to the development of stimulus thermo-responsive nanogels taking into consideration several criteria necessary for practical applications in the clinical setting, such as: (i) limiting the nanogel dimensions to less than 200 nm diameter; (ii) prolonging circulation in the bloodstream by surface modification of the nanogels, which will enhance the deliverable capability of the drug loaded nanogels to the diseased site, thus reducing the side effects; (iii) enhancing the surface functionality of the nanogels via further bioconjugation with specific ligands that can recognise receptors on diseased cells.

A common strategy to select the most suitable coated nanoparticle will be decided. Characterisation of suspension colloidal stability of coated nanoparticles in physiological media: measurements of charge and aggregation rate, and of interaction with plasma proteins, as well as MRI and hyperthermia measurements. Relaxivity and hyperthermia measurements will be performed as a function of

nanoparticle concentration to evaluate their MRI and therapeutic properties, respectively, and they will be correlated to the nanoparticle structure, physico-chemical properties and magnetic interactions.

All milestones of objective A5 (and all others) will be achieved by research activities using existing infrastructures and financial means. However, those decisions and strategies that will define the direction of the aforementioned research will be coordinated through the WG meetings of RADIOMAG, while exchange of know-how and knowledge will be ensured through STSMs. The deliverables of these objectives will take the form of joint peer-reviewed publications and joint student supervision. Finally, the co-organisation of an international meeting is envisaged.

Type of milestone & envisaged time-frame

- Action S&T Meeting: (all 4 years)
- Action Conference (4th year)
- STSMs (all four years)
- Joint Peer-Reviewed Publications (during all four years)
- Joint Student Supervision at Master's or Doctorate Level (during all 4 years)

Objective A9 – Dissemination of results to the general public

Milestone A9-1: **Dissemination of information for medical professionals and the general public, promoting magnetic hyperthermia treatment as anticancer therapy.**

In order to sensitise patients for the necessity of clinical trials with magnetic hyperthermia, it is aimed to disseminate research results to the general public. The means to achieve this objective are, for instance, informative talks in hospitals, printed brochures and internet-based resources (website, social networks) communicating the basic principles of magnetic hyperthermia, the status of current research and difficulties to overcome, as well as advantages and disadvantages of the proposed treatment.

A Working Group dedicated to the dissemination of research results will be composed of specialists with different backgrounds (bio-chemistry, physics, medicine). It is in charge of collecting the research results from network members and third parties, extracting the relevant information in order to produce dissemination material for distribution and designing an informal website.

Types of milestones/deliverables & envisaged time-frame

- Participation at S&T Meetings (during all 4 years)
- Stakeholder Outreach written input 3rd and 4th year
- Website set-up / up-date (1st semester of 1st year / all 4 years)
- Production of Dissemination Material for Distribution (3rd and 4th year)

Objective B13 – Bridging separate fields of disciplines to achieve breakthroughs that acquire an interdisciplinary approach

Milestone B13-1: **Composition of interdisciplinary Working Groups.**

The researchers participating in this network have a scientific background from different scientific disciplines. In order to motivate, to foster and to strengthen interdisciplinary working and lateral thinking, each Working Group will be composed of scientists from all participating disciplines, i.e. medicine, physics, bio-chemistry, biology, chemical engineering.

The management committee will determine and control the interdisciplinary character of the WGs. Moreover, Action Workshops and Training Schools will be organised. The latter are particularly dedicated to young scientists who will participate in different Working Groups each year. For instance, a student at doctoral level participates in the WG dedicated to hyperthermia testing during the first year, during the second in the WG dedicated to coating/fabrication/targeting and during the 3rd year in the WG dissemination. RADIOMAG Training Schools in collaboration with external experts will provide the necessary scientific background for a quick familiarisation with the corresponding topic.

Types of milestones/deliverables & envisaged time-frame

- Action S & T Meeting for WG and STSM committee (all four years)
- Action Workshops (1st, 2nd & 3rd year)
- Training Schools (beginning 2nd and 4th year)
- Achievement of Specific Network Features in terms of WG composition (during all four years)
- Input for the Formulation of Framework Programme Calls (3rd year)

D. ACTION STRUCTURE AND PARTICIPATION – WORKING GROUPS, MANAGEMENT, INTERNAL PROCEDURES

In order to achieve efficiently the objectives of this COST Action, its structure is based on three pillars: the Management Committee (MC), the scientific Working Groups (WG) and the public relations WG.

The MC will be responsible for overall organisation and management guaranteeing the correct implementation of the Action according to COST rules. Because the number of MC members may increase during the Action's lifetime and may become large, a Core Group (CG) will be established. During the 1st MC Meeting, CG members, WG leaders, the MC Chair and MC-Vice Chair will be elected by the MC. Moreover, it will be debated about the distribution of financial means, the potential adaption of the Action strategy, WG composition and work plans to ensure successful networking. Certain administrative duties will be delegated to individual MC members, such as the coordination of gender-balance, STSM and training coordination as well as the involvement of Early Stage Researchers (ESR). Chair and Vice Chair will report to the COST office on the Action's progress and the financial issues, after approval by the MC. The annual MC meetings are dedicated to review the Action strategy, to approve internal and external reports and to vote about upon suggestions of the CG for new members possibly also from International Partner and Near Neighbour Countries and the assessment of financial means.

The CG, which includes all WG leaders and the persons responsible for STSM managing, ESR participation and gender policy, will review the WG composition and, emphasising hereby that scientists of different background are present in each WG, that there are enough Early Stage Researchers and that the gender policy is respected. It will further propose to the MC new Action members, MC observers and external experts to be invited for the Training Schools / Action Workshops, will control the compliance of the work plan according to the milestones and deliverables foreseen, will report yearly to the MC on the achieved activities and will make recommendations to the MC about future visions of this COST Action. The CG will discuss via a web-based application (e-conference) every two months about administrative tasks and the scientific progress. The latter shall guarantee inter alia the strong exchange of unpublished aspects of knowledge. The CG also will set up the agenda for joint WG workshops and STSMs, the latter in coordination with a STSM manager. The CG will report regularly on their bi-monthly e-conferences to both the Chair and the Vice Chair.

There will be four scientific WGs:

- **WG1 "Physical Chemistry of fabrication / coating / targeting"**
- **WG2 "Physical aspects of hyperthermia: standardisation and testing"**
- **WG3 "Combined radiosensitisation and magnetic thermotherapy: pre-clinical and clinical aspects"**
- **WG4 "Instrumentation: thermometry aspects and development of hyperthermia set-ups"**

The scientific WG leaders are responsible for coordination of research in their individual WGs according to the Action's research objectives. They will discuss with other WG members the optimal share of research infrastructure, the involvement and integration of nationally / regionally funded activities into this COST Action, the execution and of individual joint research tasks according to the milestones. The scientific WGs will also be in charge of Joint Student Supervision (at Master's or Doctorate Level) and attracting Early Stage Researchers. The latter is of particular importance because there are only two of them in the network of proposers. On demand of the WG 5 "Public Relations", the scientific WGs will provide specific input for documents intended for the broad public and for stakeholders.

The interaction between the scientific WGs will be fostered by joint scientific WG meetings each year, bi-annual Training Schools, STSMs, Action Workshops and Joined Peer-Reviewed publications and moreover by joint WG doctoral theses with interdisciplinary flair.

- **WG5 "Public Relations"**

This WG is in charge of internal and external public relations. It is responsible for developing and maintaining the website of the Action, the collection of information from the scientific WGs in order to produce dissemination material. It will present the network as a whole at international conferences, organise public talks and establish contacts with hospitals to win over new community members, international experts and stakeholders (e.g. industry, patient organisation, regulatory bodies) and seeks to fathom possible collaboration with other COST actions (e.g. TD1004, TD1205). The contact with hospitals is of particular interest because the current network of proposers consists only of three clinicians, but a broader involvement of medical specialists (e.g. oncologists) is highly desirable as they are possible end-users of the applications developed under the coordination framework of this Action.

WG5 will obtain a report from each Action event and will stay in contact with the scientific WG leaders (e.g. via the bi- monthly CG e-conferences, joint WG meetings) to ensure the correct public dissemination of scientific progress. WG5 will give general feedback (electronically) to the scientific WGs, look for new conference participation, international funding opportunities, organise certain scientific dissemination (e.g. editing special volumes on the Action topic in internationally recognised journals), provide an exchange platform for ideas and knowledge via a secured website access and keep an overview about new scientific publications concerning the topic of the Action.