Dartrix

DARTRIX – DARPin Targeted Magnetic Hyperthermic Therapy for Glioblastoma
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PROJECT FINAL REPORT

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1. Final publishable summary report

1.1 Executive summary

DARTRIX, 'DARPin Targeted RX (therapy)' is a multidisciplinary collaborative project aimed at developing targeted hyperthermia as a treatment for glioblastoma. Heat is directly toxic to cancer cells and can also help activate the immune system within the tumour microenvironment. The approach is innovative and there is great need for new treatments as glioblastoma is virtually incurable and most patients die within 12 months of diagnosis. The project employed Designed Ankyrin Repeat Proteins (DARPins) which are small, stable, non-immunoglobulin human protein scaffolds that bind specific targets with exceptionally high affinity. The goal was to couple DARPins to superparamagnetic iron oxide nanoparticles (SPIONs). SPIONs can generate heat when stimulated by an alternating magnetic current and, by functionalisation with DARPins, the SPIONs would have potential to bind selectively to tumour cells.

The consortium brought together a range of diverse skills to achieve the ambitious aims of the DARTRIX project. DARPins were generated for glioblastoma-related targets and the lead DARPin was manufactured to standards of GMP (good manufacturing practice) with a unique cysteine for site specific attachment to SPIONs. A new range of GMP compliant SPIONs were created with excellent heating properties and conjugation strategies were optimised to link the DARPins to these particles. Full toxicity testing was conducted on the lead SPIONs which were shown to be well tolerated, with no adverse effects either systemically or at the site of administration. Methods were developed to reduce unwanted uptake of SPIONs by the reticuloendothelial system. DARPins were engineered with long hydrophilic random coil polypeptides, creating second-generation DARTRIX particles with superior specificity for targeting cancer cells. Furthermore, employing immunocompetent pre-clinical models of glioblastoma, the consortium demonstrated that the GMP-compliant SPIONs generated effective and localised heat in tumours using a bespoke medical device to induce magnetic alternating current hyperthermia (MACH). A scaled up MACH system was developed for clinical use and tested in a hospital environment. This scaled-up MACH system is now operating at clinically relevant field strengths and is undergoing testing to achieve CE Marking. Finally, novel ways to track DARTRIX particles *in vivo*, and to measure the biological response to particle-mediated hyperthermia within the tumour microenvironment, were developed in pre-clinical models.

The results generated during the DARTRIX project will contribute substantially to knowledge in the scientific field in addition to providing a sound platform to facilitate a future DARTRIX first-in-human trial.

1.2 Summary description of project context and objectives

1.2.1 Context (State of Art at the project start, Key challenges)

DARTRIX is a multidisciplinary collaboration to develop therapeutic, biocompatible, targeted SPIONs for localised hyperthermia treatment of cancer. In current clinical practice, SPIONs are used as contrast agents in magnetic resonance imaging (MRI). However, when subjected to an alternating magnetic field, SPIONs can readily heat to temperatures above 42°C. The aim of the DARTRIX project was to harness the heating potential of SPIONs to develop an innovative new cancer therapy that would safely deliver effective localised hyperthermia within the tumour. Challenges were to generate biocompatible GMP-compliant SPIONs that would be safe and effective in vivo and to build

a clinical-grade MACH system capable of generating therapeutic heat in patients. Because SPIONs are not tumour specific, the ultimate goal was to target them to cell surface proteins such as epidermal growth factor receptor (EGFR), a widely recognised tumour target that is often overexpressed in glioblastoma. DARPins were chosen as targeting agents due their favourable affinity, stability, small size, economy of production and amenability to protein engineering. The DARTRIX project focused on treatments for glioblastoma (WHO grade IV), a highly infiltrative, rapidly progressive primary brain tumour that continues to be associated with a poor prognosis. There is no effective standard treatment for management of patients with recurrent/relapsed glioblastoma and an urgent need for new treatment approaches.

1.2.2 Objectives of the project

The DARTRIX project aims to develop a new, safe and efficient approach to targeted hyperthermia treatment by creating and applying a cancer-targeted SPION or 'DARTRIX particle', in parallel with a custom-made MACH system to deliver the alternating magnetic field. The DARTRIX particle should be clinically compatible, reproducible, targetable to appropriate tumour areas, imageable in patients, non-toxic prior to MACH activation and able to produce measurable localised heat upon activation. A successful outcome of the DARTRIX project would form a foundation for development of new treatments for glioblastoma and eventually other diseases.

1.3 Description of the main S&T results/foregrounds

1.3.1 UZH

The challenge of the project was to couple targeting moieties to magnetic nanoparticles, which would survive heating, be compatible with many kinds of coupling chemistry, show extreme specificity and be non-immunogenic, allow the attachment of additional labels and help the final particles to be resistant to uptake by the reticuloendothelial system (RES).

We chose to use Designed Ankyrin repeat Proteins (DARPins) as a basis. DARPins are small, non-immunoglobulin human protein scaffolds that have great potential to form the targeting moiety of clinically effective therapeutics. UZH has developed a strategy to build combinatorial libraries of repeat proteins by extracting sequence and structural information from compatible natural repeats to design a consensus amino acid sequence motif encoding self-compatible repeat modules. Indeed, DARPins are ideally suited for this particular therapeutic purpose — they are readily generated to bind specific targets with exceptionally high-affinity and they are remarkably stable, even at high temperatures. Furthermore, DARPins can be manufactured in gram quantities using simple bacterial expression systems amenable to GMP production and to economic scale-up.

Since the project needed DARPins to develop treatments for glioblastoma, the epidermal growth factor receptor (EGFR) was the most obvious target. EGFR is overexpressed in 50-60% of glioblastomas, the prototype anti-EGFR DARTRIX particle was functionalised using high affinity EGFR1-binding DARPins.

The anti-EGFR1 DARPin chosen was one which had no pharmacological activity (it did not compete with EGF on the receptor). It could be well expressed in *E. coli*, and its expression could be well

transferred to *P. pastoris* in a GMP process. The DARPin carried a cysteine for versatile coupling to suitably modified magnetic nanoparticles.

Importantly, rigid quality control of the yeast-produced DARPins showed that they are fully functional and have the exact expected mass, and the previously established binding constant, as measured by surface plasmon resonance.

Important considerations in engineering the DARPins were to ensure target recondition when the DARPins were coupled to particles and to minimise uptake by RES and local macrophages. For this purpose, DARPins were engineered to be equipped with a long polar extension, either negatively charged, or uncharged (between 300 and 900 amino acids). A highly efficient expression and purification method was established for these constructs, and minor impurities were removed, using two purification tags, one of them cleavable and a very inexpensive affinity column (itself exploiting DARPins).

The so purified DARPins with cysteines and long polar extensions could be efficiently coupled to magnetic nanoparticles and were shown to specifically recognise the target on the surface of tumour cells.

In addition, DARPins that bind new targets relevant for glioblastoma were generated from the library by an extensive series of ribosome display experiments and subsequent characterization. E.g., a series of anti-cMET DARPins were generated and characterised in detail. They were shown to cover many different epitopes on cMET on different domains, show high specificity, react on cells and show low nanomolar KDs. Using the technologies established for the EGFR binders, which are completely generic, they can now be manufactured in very similar formats.

1.3.2 MICROMOD

1.3.2.1 Development of DARTRIX particles

MICROMOD has introduced a new type of iron oxide dextran composite nanoparticles (perimag®) into the DARTRIX project. These new particles are produced in a clean-room facility for GMP compliance and show favourable and consistent heating properties under the conditions of the MACH system (RCL). The surface chemistry of these particles was tailored for site specific conjugation of DARPins with a terminal thiol group by introduction of carboxylic acid or amino groups to obtain DARTRIX particles. For surface functionalisation with amino groups, the dextran surface of the particles was cross-linked with epichlorohydrin followed by reaction with ammonia. Amino group density on the particle surface was altered by changing the general reaction parameters. Thus, DARTRIX particles with high and low density of amino groups were prepared to study the influence of the density of functional groups and surface charge on the cell-uptake of the final DARPin conjugate at UCL (Figure 1). The surface potential of the functionalised particles was characterised by electrophoretic measurements of the zeta potential of the particles in dependence on the pH value. The DARTRIX particles with COOH groups on the surface possess a negative zeta potential over the whole pH range. The increase of the density of amino groups is accompanied by a shift of the zeta potential into positive direction (Figure 2).

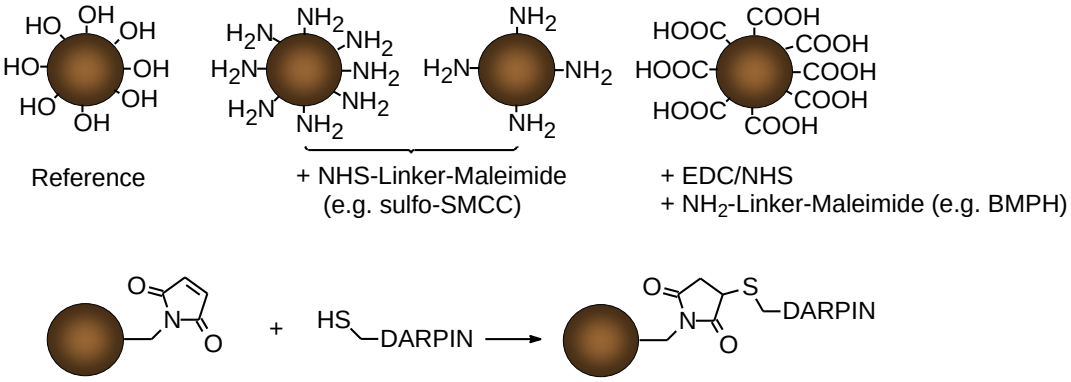

Figure 1: Surface modifications of perimag® particles for DARPin conjugation.

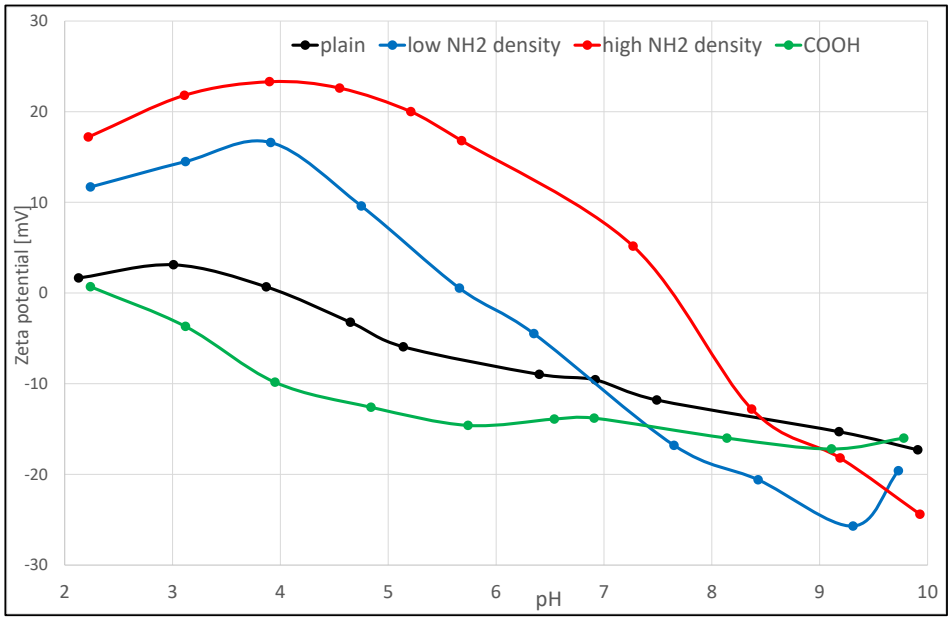

Figure 2: Zeta potential-pH functions of perimag® particles with different surface functionalities.

Different strategies were established to conjugate DARPins on aminated or carboxylated particles. The aminated particles were functionalised with maleimide groups for reaction with the thiol group of the DARPins. Hereby the length and flexibility of the spacer between particle surface and terminal maleimide groups was varied. The performance of rigid cyclohexyl spacers and flexible PEG spacers with 6, 12 and 24 ethylene glycol units was compared. Furthermore the density of DARPins on the particle surface was varied (Figure 3). For DARPin conjugation on carboxylated particles, the COOH groups were activated with carbodiimide to facilitate reaction with a PEG spacer that contains terminal amino and maleimide groups (Figure 4). All conjugation strategies were performed with the specific E69 DARPin and the He-G3 DARPin as reference.

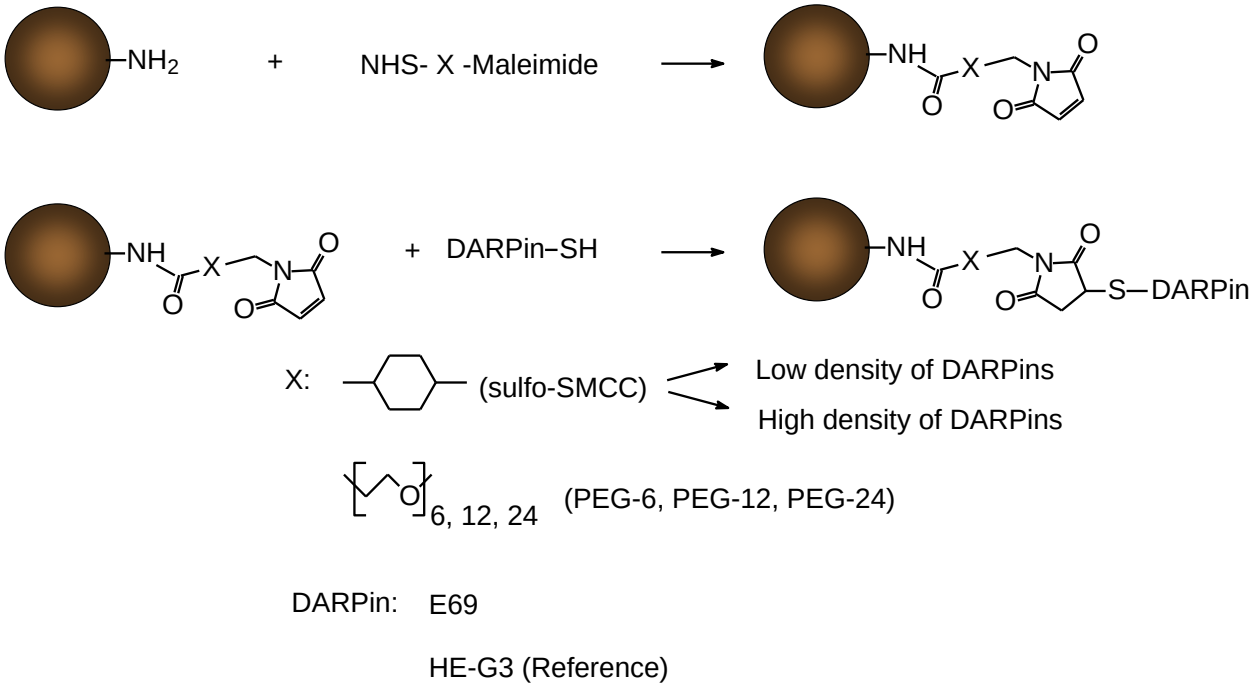

Figure 3: DARPin conjugation on aminated perimag® with different spacers.

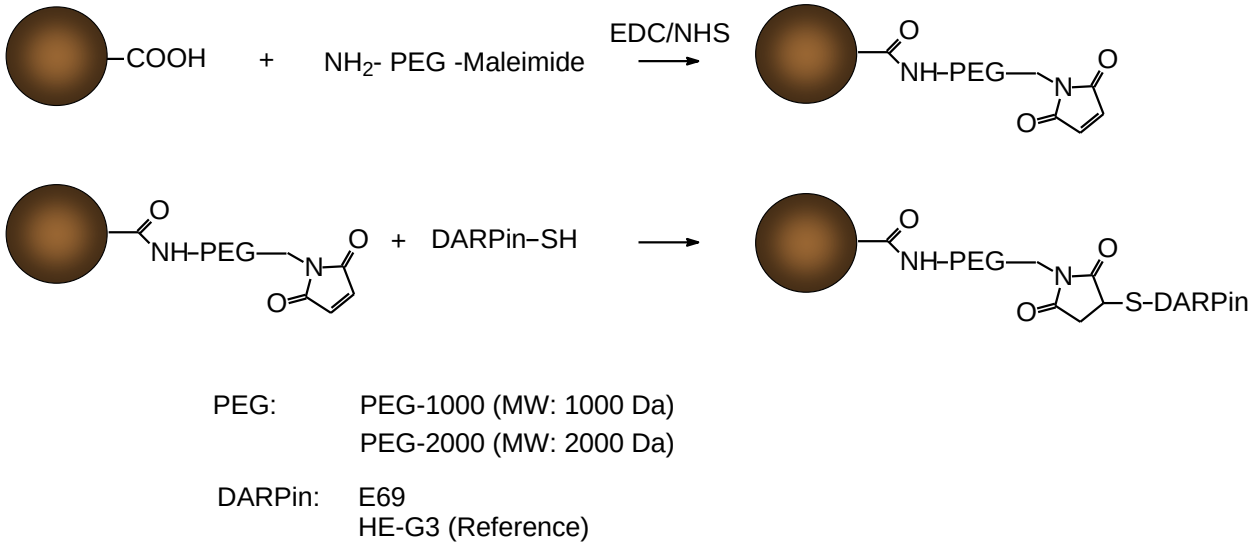

Figure 4: DARPin conjugation on carboxylated perimag® with different spacers.

UZH has introduced longer spacers between the bioactive E69 unit and the terminal SH group to achieve a higher flexibility of the DARPins on the particle surface for an improved specific cell binding of the DARTRIX particles. MICROMOD has obtained these new PAS- and XTEN-DARPin derivatives from UZH (Figure. 5, Table 1) for conjugation to perimag® particles. The PAS-DARPins were prepared with a short and long flexible chain between the DARPin and the terminal cysteine.

The XTEN288-DARPins have a higher number of negative charges compared to the XTEN864-DARPins. Each PAS- and XTEN-DARPin was provided as specific E69 DARPin and as Off7 reference DARPin. These new DARPins were conjugated to maleimide functionalised perimag® particles according to Figure 6. The size distribution and the zeta potential of all DARTRIX particles were measured by dynamic light scattering. The DARPin content of the DARTRIX particles was determined by a modified BCA (bicinchoninic acid) assay. The physico-chemical data of the obtained DARTRIX particles are summarised in Table 2. All DARPin particle conjugates show a good colloidal stability and were provided for cell-uptake studies at UCL In addition the maleimide functionalised particles were reacted with cysteine as reference for the *in vitro* cell uptake studies at UCL.

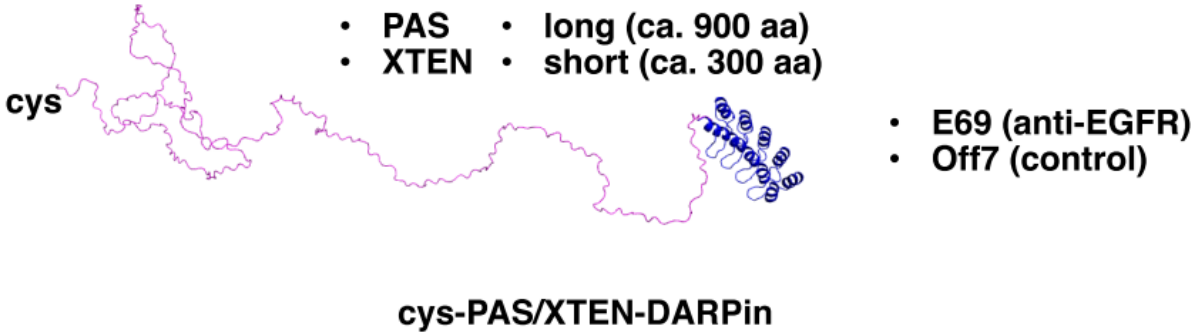

Figure 5: Scheme of new generations of PAS- and XTEN-DARPins from UZH.

Table 1. Overview on new PAS- and XTEN-DARPins and their corresponding positive and negative charges.

Construct	pI	Negative charges	Positive charges
c-PAS300-E69	5.18	34	14
c-PAS300-Off7	5.26	27	11
c-PAS900-E69	5.18	34	14
c-PAS900-Off7	5.26	27	11
c-XTEN288-E69	4.31	82	14
c-XTEN288-Off7	4.25	75	11
c-XTEN864-E69	3.88	178	14
c-XTEN864-Off7	3.8	171	11

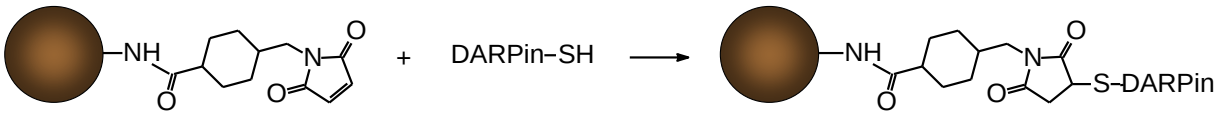

Figure 6: DARPin conjugation of maleimide functionalised perimag®.

Table 2. Physico-chemical properties (size, zeta potential and protein concentration) of DARTRIX particles with new PAS- and XTEN DARPins on the surface.

Particle surface	c (Fe) [mg/ml]	Z_{AVE} [nm] (PCS)	PDI	ZP [mV]	c (DARPin) [µg/mg Fe]
c-PAS300-E69	4,6	142	0.24	-13,4	18
c-PAS300-Off7	4,7	144	0.21	-13,5	16
c-PAS900-E69	4,4	139	0,22	-11,9	18
c-PAS900-Off7	5,8	142	0,21	-12,8	16
c-XTEN288-E69	4,8	140	0,22	-20,4	21
c-XTEN288-Off7	5,1	146	0,22	-20,4	21
c-XTEN864-E69	5,6	146	0,23	-20,0	17
c-XTEN864-Off7	5,5	143	0,20	-22,6	19
Cysteine (reference)	4,8	137	0,21	-15,0	12
NH_2 (reference)	8,2	142	0,24	+0,02	0

All new DARTRIX particles had a hydrodynamic diameter of about 140 nm. The DARPin concentration of the particles lies in the range of 16-18 µg/mg Fe for the PAS-DARPin derivatives and of 17-21 µg/mg Fe for the XTEN-DARPin derivatives.

The conjugation of aminated perimag® particles with PAS-DARPins leads to a significant shift of the zeta potential from about +15 mV at pH=7 to negative values in the range of -10 to -15 mV (Figure 7). The zeta potential of all XTEN-DARTRIX particles is more negative than that of the PAS-DARTRIX particles. This correlates well with the higher number of negative charges of XTEN-DARPins in comparison to PAS-DARPins (Table 1, Figure 8). The isoelectric point of all new DARTRIX particles lies at pH=4.

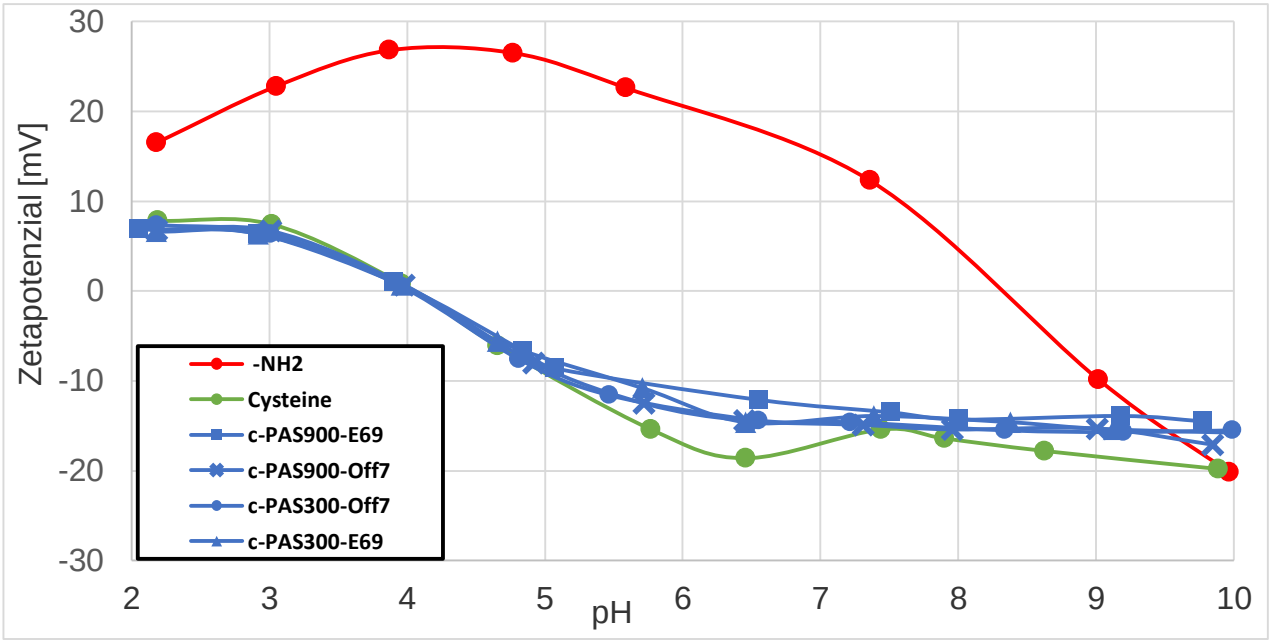

Figure 7: Zeta potential-pH functions of PAS-DARPin conjugated perimag® particles in comparison to the aminated precursor particles.

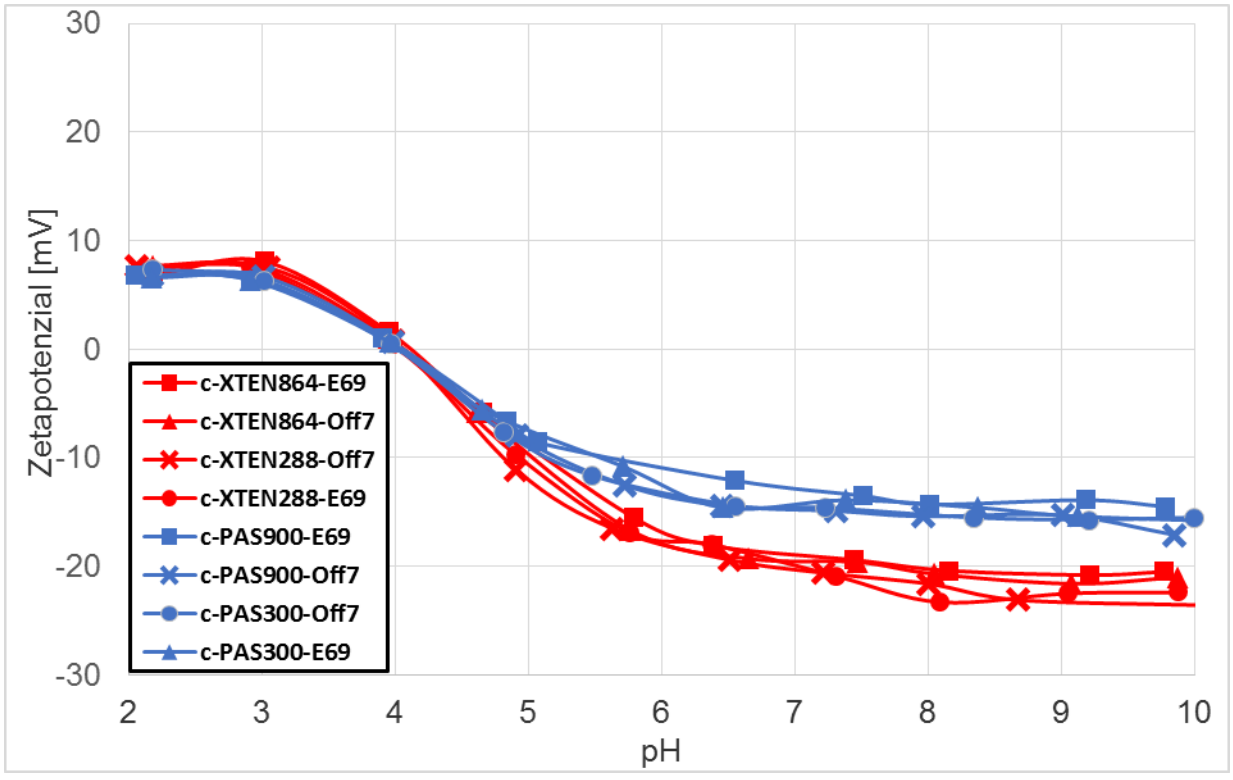

Figure 8: Zeta potential-pH functions of PAS- and XTEN-conjugated perimag® particles.

All PAS- and XTEN-conjugated perimag® together with the cysteine-labelled reference particles were sent to UCL for *in vitro* cell uptake studies. Initial results from UCL demonstrated a high specific binding of PAS-E69 and XTEN-E69 particles in comparison to the corresponding Off7 conjugates and the cysteine reference.

DARTRIX particles with different iron concentrations, surface functionalisations and formulations were delivered in sufficient amounts for toxicity studies to UCL and TOPASS. The colloidal stability of these particles was investigated for particle formulations in water and artificial cerebrospinal fluid (aCSF). Dynamic light scattering measurement did not show any significant changes of the size distributions over a period of 6 months. The heating parameters of these particles were measured at RCL. The Intrinsic Loss Power (ILP) values of all particle types that were delivered for toxicity studies are in the range of 6.3 - 6.7 nHm^2/kg.

1.3.2.2 Quality management system

MICROMOD has been registered as an ISO 9001 and ISO 13485 certified company. This registration includes all organisation processes applicable to purchase order processing, manufacturing, testing and quality assurance. These certificates attest MICROMOD a quality management system which allows us to be able to produce and develop medical products according to applicable legal or regulatory requirements respectively. These legal requirements are effective in addition to the technical requirements of the products.

The legal requirements for manufacture and distribution of medicinal products and active substances using as starting material are derived from the EU GMP guidelines. Part I of GMP guide describes GMP principles for the manufacture of medicinal products, Part II for active substances used in starting materials and Part III GMP related documents. The GMP guide is supplemented by a series of annexes. Compliance with the guide is regulated in various national laws (AMG, AMWHV, national and international pharmacopoeias). The requirements to investigational medical products are described in Annex 13 of the GMP guide.

MICROMOD was audited by UCL for verification of compliance with ISO 9001:2008 and ISO 13485:2015 and adequate good laboratory practices for manufacture of IVD. The quality system and operational activities for production, laboratory, QC & support operations in the context of manufacture of SPION for first DARTRIX clinical trial were in the scope of audit. QMS and facilities of MICROMOD were found to be compliant with current regulations and standards required for the manufacture of IVD.

1.3.3 RCL

1.3.3.1 MACH system

The MACH system (Magnetic AC Hyperthermia) was developed as a scalable and modular system that could be used for *in vitro* and *in vivo* as well as clinical applications. One of the key features of the system is the ability to self-tune to the resonance of the field applicator, in this case the head coil, which allows high efficiency of the transfer of energy from the system to the coil. Further to this, the self-tuning mechanism allows positioning of the coil to ensure maximum field exposure is applied to the region of interest i.e. site of the magnetic nanoparticles. Throughout the project the core PCB (printed circuit board) underwent 18 revisions, with each iteration making improvements on performance, scalability and safety. Figure 9 and Table 3 highlight some of the major revisions to the PCBs along with maximum AC magnetic field achieved with the clinical coil.

Table 3: MACH system PCB progress.

Revision	Date	Comments	Clinical Field (A/m)
0.1	Q1 2012	Proof of concept with a single drive chip	180
1.0	Q2 2012	First single PCB with 4x drive chips	670
9.3	Q1 2013	Optimised single board with 6x drive chips	1680
10.0	Q4 2013	Demonstrated scalable system with 2x PCBs working in parallel	2480
14.0	Q4 2014	4x PCBs housed in 19" rack for clinical system. Now working at a potentially usable clinical field. Added safety relays to cut-out in event of certain failure modes.	2940
16.0	Q4 2015	4x PCBs with similar set-up as 14.0 but with optimised components to achieve higher fields	4010
17.0-18.0	Q4 2016	6x PCBs. Final revision before undergoing EMC tests.	5000-6000

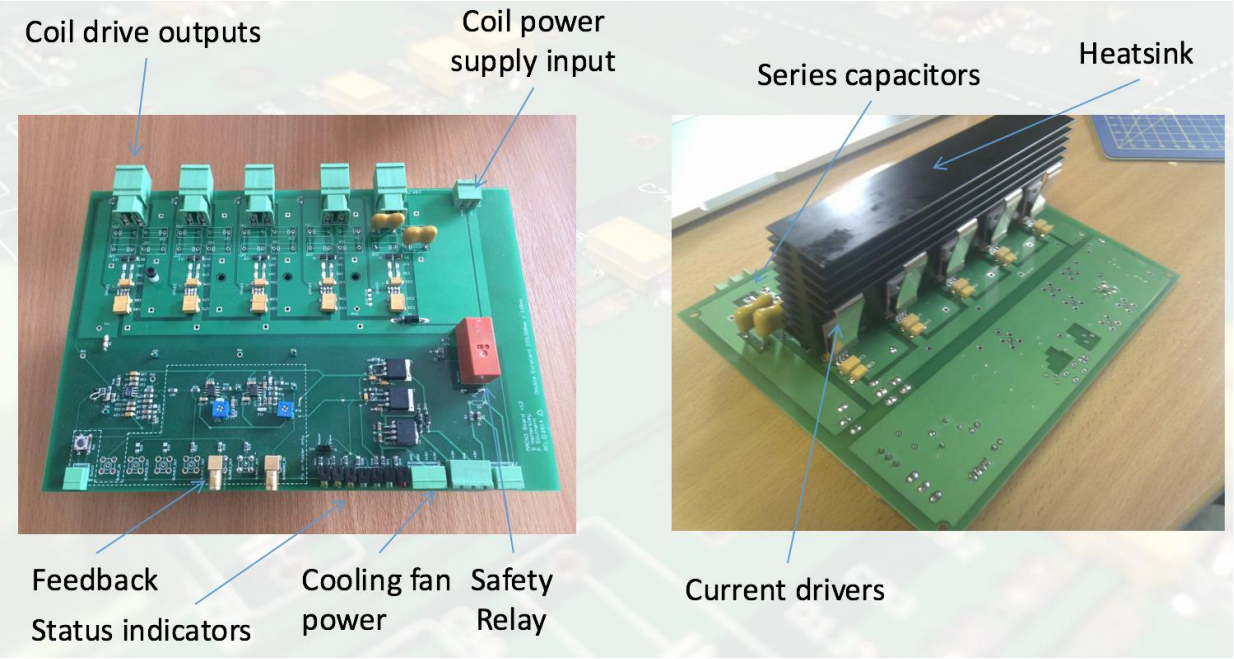

Figure 9: MACH system PCB progress.

1.3.3.2 *In vitro* / *in vivo* MACH development

Several lab scale systems were built for the DARTRIX project to undertake particle characterisation and small animal work, both of which used single PCB set-ups to generate the magnetic field. The *in vitro* / characterisation system utilised a multi-turn solenoid style coil that was capable of generating fields up to 15 kA/m at ~1MHz, whereas the *in vivo* / animal system used a split pancake Helmholtz style coil with a maximum field of 6 kA/m at 800 kHz. The open style *in vivo* coil allowed direct line of sight for infra-red thermography, something that other hyperthermia systems struggle with due to lack of access, particularly when using solenoid coils. Although the *in vivo* coil could only produce just under half the field of the *in vitro* model, considerable effort was made to ensure that the fields would be sufficient for animal experiments (see section on bioheat modelling) as well as providing as uniform field distribution as possible over a large treatment volume. In the case for the *in vivo* coil, the field homogeneity varied by only 10% over a 20x20x20mm volume, as shown in Figure 10 and 11.

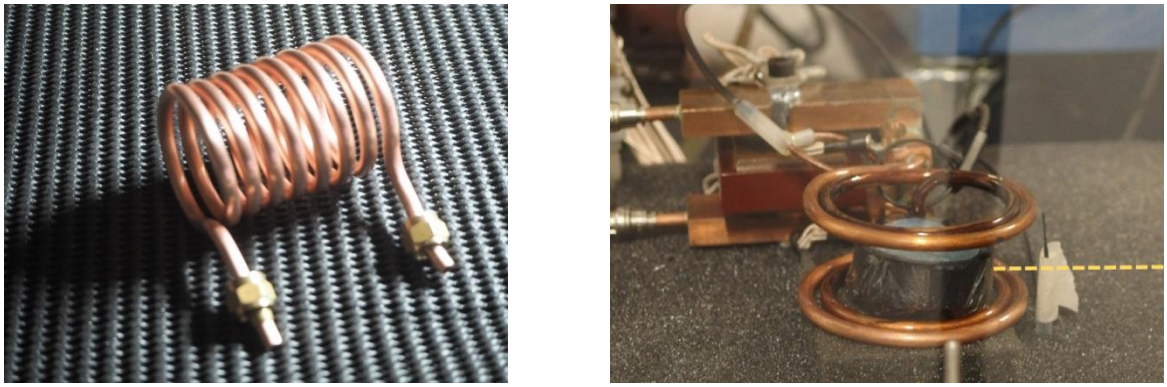

Figure 10: (left) solenoid style coil and (right) split pancake Helmholtz coil.

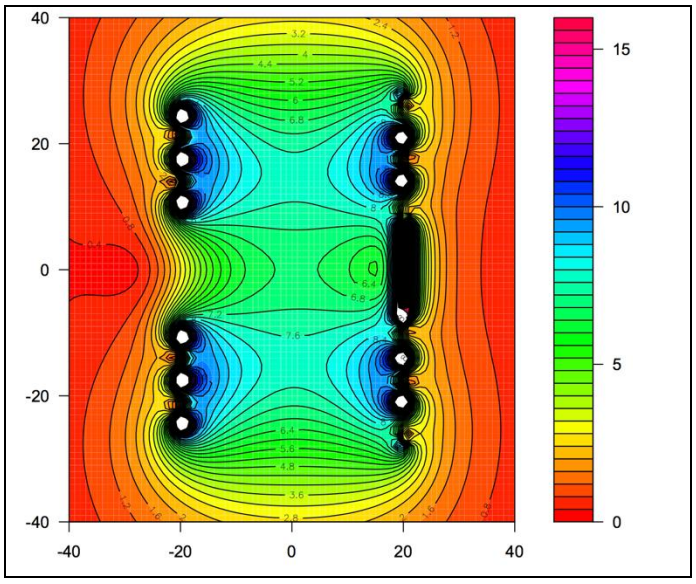

Figure 11: Field distribution for split pancake Helmholtz coil for *in vivo* / animal use.

1.3.3.3 Clinical MACH development

The primary goal for device development was to build and demonstrate a magnetic hyperthermia system suitable for a clinical environment capable of generating an alternating magnetic field of 5 kA/m. The constraints of development lie mainly in portability and the ability to run the system using power from a 240V 13A socket i.e. not exceeding 3 kW of power. Early models showed that the scalability of the MACH PCBs would allow currents of 800-1000A to be generated in a single turn head shaped coil that would generate the required 5 kA/m. This required the use of 6x PCBs utilising the full 3 kW from a power socket. Although this style of coil would have sufficed there would have been little headroom to increase the field if required unless more PCBs were used along with a second power supply (thus limiting the portability of the system). To overcome this problem, a novel shaped coil was designed which was named the "dished elliptical coil". This coil was a double turn ellipse that was dished over a cylinder to form a concave shaped coil that could then be positioned over the patient's head, rather than round it. The added benefit here was the coil could now "project" the field more efficiently from the central part of the coil than a circular coil (Figure 12).

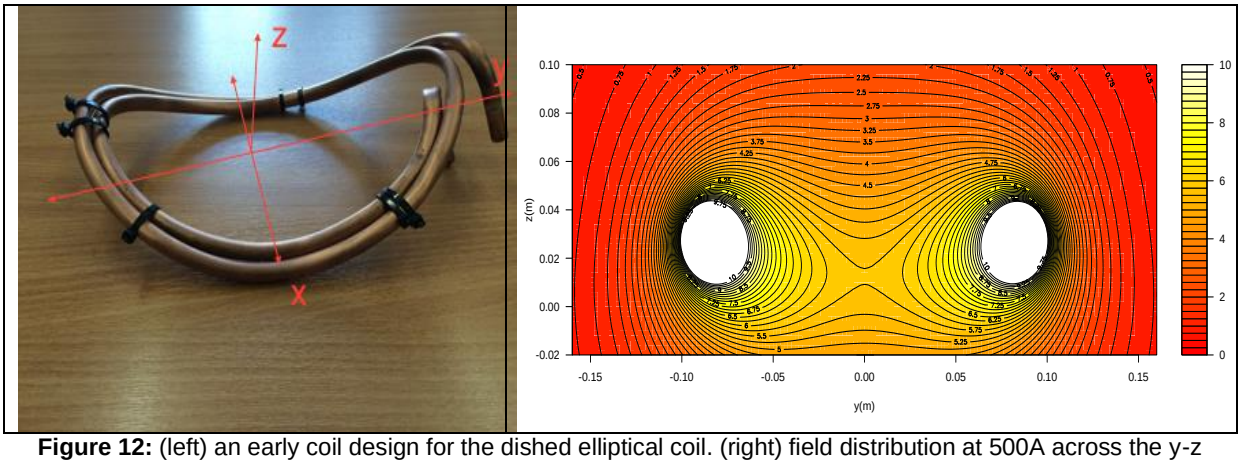

Figure 12: (left) an early coil design for the dished elliptical coil. (right) field distribution at 500A across the y-z plane.

This design also allowed for a second turn due to the decrease in total inductance per turn when compared with the single turn circular coil and thus reducing the required current to generate 5 kA/m from ~1000A to 600A. This structural change in coil design would permit some level of flexibility if higher fields would be required (although other considerations such as heat dissipation and high voltages would need to be addressed).

Another consideration to be made was in reducing the overall resistance of the coil. The impact of driving high frequency currents in a conductor is overcoming a problem called the skin effect. In short, the depth in which current can travel within a conductor is inversely proportional to the square root of the frequency. Therefore, the higher the frequency of the applied current, the less area the current can travel through the conductor i.e. less efficient. For a current at 300 kHz, the skin depth would be ~120 µm. It was therefore important to use a special type of wire called Litz wire that has hundreds of individually insulated strands per bundle.

The final version of the dished elliptical coil had an outer major axis of 19cm and an outer minor axis of 16cm dished over a 30cm diameter cylinder with a total number of 28 litz bundles each with 420 strands of wire i.e. 11,760 strands of individually insulated wires around the coil.

Figure 13: (left) Example of litz wire termination for head coil. (right) demonstration of mounted dished elliptical coil.

1.3.3.4 Frequency Tracking

One possible advantage of the MACH system over a conventional hyperthermia system is the novel technique through which the system "tracks" the resonant behaviour of the coil. Firstly, this allows the system to precisely lock-in to resonant frequency and run at high efficiency without the need of user intervention to tune the drive circuit. Secondly, changes in the resonant frequency can be used to provide feedback to the user. It was noted that under controlled laboratory conditions it was possible to indirectly measure the temperature of magnetic nanoparticles through a change in resonant frequency. Further to this any sharp change in resonant frequency when positioning the coil can be linked to mass of magnetic material.

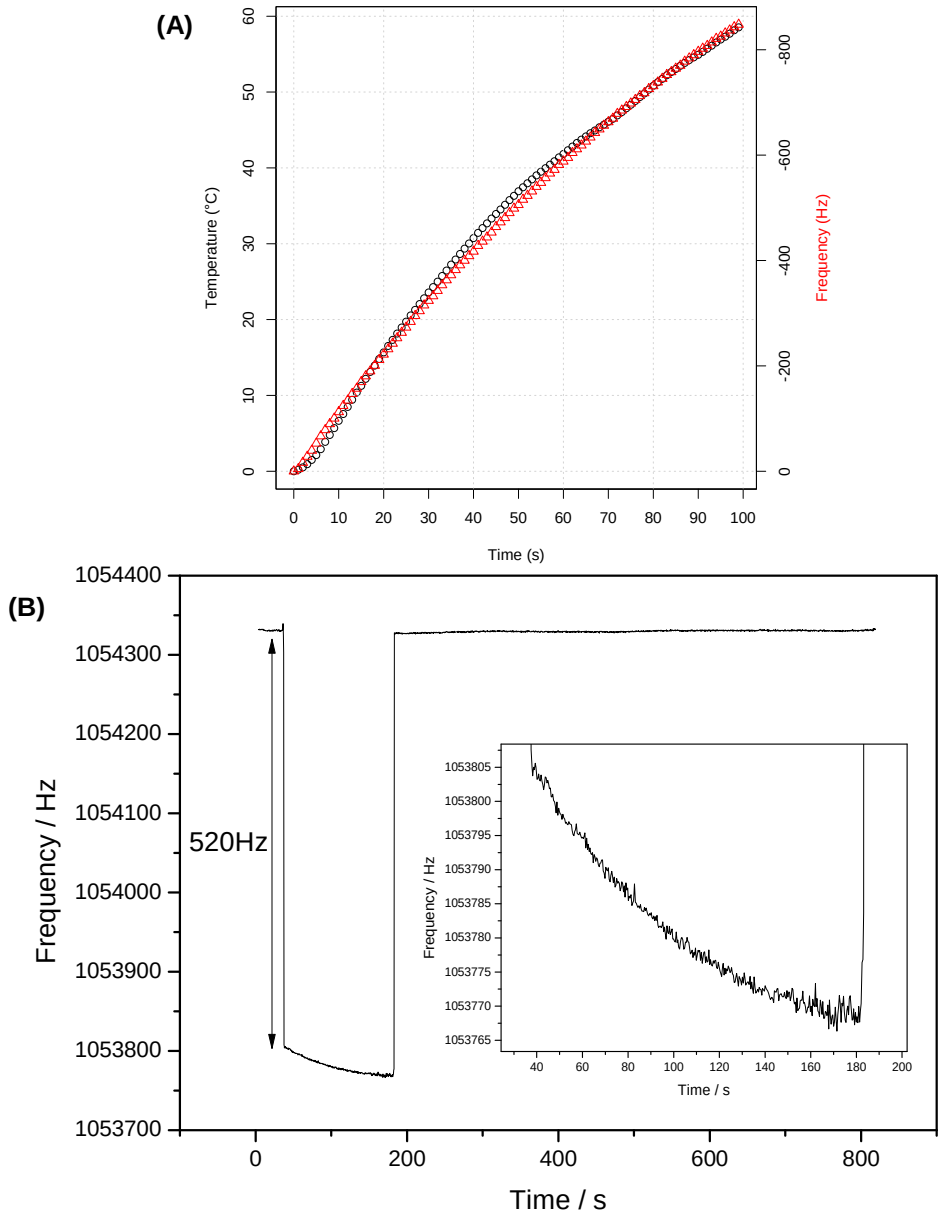

Figure 14: Top **(A)** frequency tracking vs temperature using fibre optic temperature probes, note the linear scaling relationship. Bottom **(B)** upon inserting a sample into the coil there is a sudden drop in resonant frequency due to the change in inductance of the resonant circuit. This shift in resonance can be linked to the mass of magnetic material introduced into the system and for the *in vitro* characterisation system there is potential to measure the mass of Fe-oxide nanoparticles with 10µg resolution.

1.3.3.5 EMC tests

With the MACH system able to operate at clinically relevant fields, the next steps will be to run a series of stability tests and fully document all aspects of the system which will include electromagnetic compatibility (EMC) testing with the final aim of obtaining certification that complies with the EN60601 standard for Medical Electrical Equipment and Systems. The goal of EMC is to make sure the equipment will function properly in an electromagnetic environment with emissions not exceeding existing standards as well as the equipment not being prone to failure due to radio frequency interference. Although there are over 70 standards that the MACH system will need to comply with in order to be CE-ready, we have been confident from early tests of the system beside medical equipment (ECG, Blood pressure and pulse monitors) with no signs of interference. Further to this the MACH system is purely an analogue system (in electronics terms) with no software or digital decisions required to control the system. From this perspective, it has been suggested that steps towards a CE-ready system will be easier to achieve.

Preliminary EMC measurements were performed at ETS (Electromagnetic Testing Services Ltd) on an operating MACH system driving the two-turn head coil. The system was running at 300 A peak, drawing about 500 W from the main supply (a higher power was not possible due to limitations in cooling facilities).

Emissions from systems are divided into two categories, conducted and radiated. Conducted emissions are measured in a lower frequency range, 150 kHz to 30 MHz, where the wavelengths are generally too long for radiation to be a problem. Radiated emissions are measured in a higher frequency range, 30 MHz to 1000 MHz.

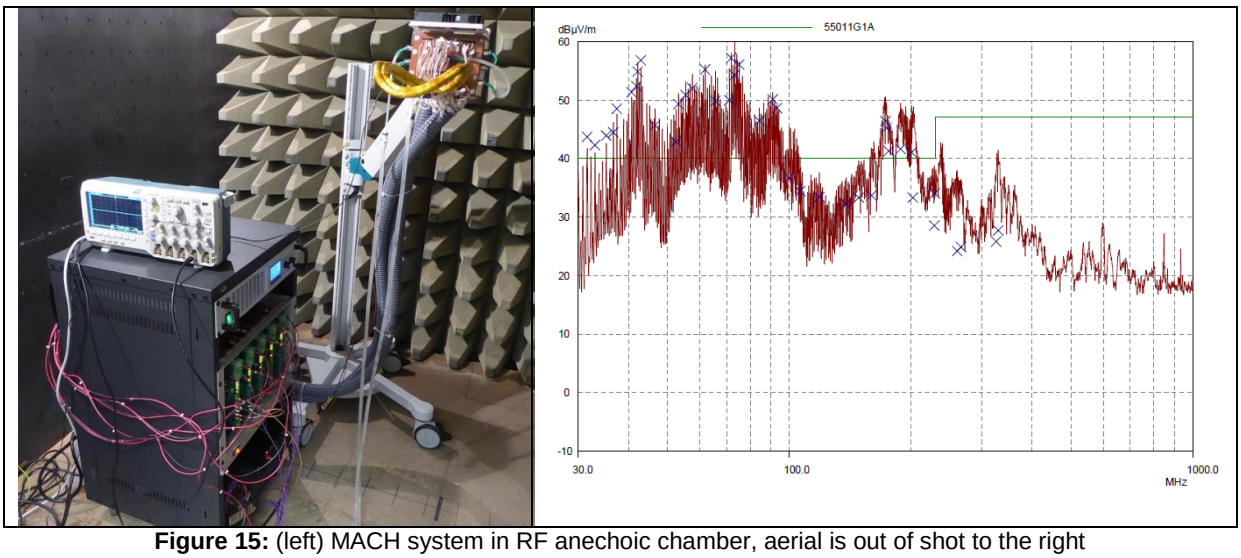

Figure 15: (left) MACH system in RF anechoic chamber, aerial is out of shot to the right (right) radiated emissions result from MACH system.

The system exceeded the limits for both radiated and conducted emissions. But given the lack of EMC precautions currently implemented in the prototype circuit, and the power levels being handled, the excess is acceptable. For instance, the highest radiated peak seen was around 57 dBµV/m at 3 m, which is equivalent to 0.15 µW if radiated isotropically, compared to the 500 W that the system is handling. It is also encouraging to note the relatively low level of emissions at the resonant fundamental (around 367 kHz), and at its low order harmonics, despite the huge currents we are

generating at that frequency. These tests have allowed us to make a number of changes to the circuits to reduce the emissions and the modifications will be tested in-house with our own EMC test equipment to ensure regulations are met before applying for CE certification.

1.3.3.6 Bioheat thermal models

Aside from development of the hardware, RCL has put considerable effort into implementing bioheat models to estimate the required doses of magnetic nanoparticles for pre-clinical and clinical set-ups (Hergt & Dutz, (2007). The basic model, as shown in Equation 1, has been modified to take into account the effectiveness of the heating of the magnetic nanoparticles (given by the ILP/SLP), the spread of material at the injection site and the heat conductivity of the tissue. Figure 16 shows a vast difference between achievable temperature rises for animal and clinical models using perimag® magnetic nanoparticles which is linked to the volume of injected magnetic material.

$$\Delta T = \mathrm{SLP}\cdot c\cdot R^2/(3\lambda) \quad (\lambda = 0.64\,\mathrm{WK^{-1}\,m^{-1}}$$
is the heat conductivity of tissue).

Equation 1 - Bioheat equation to determine the temperature rise ΔT, where SLP is the specific loss power of the injected material (W/g), c is the concentration of material in the tissue (mg/mL) and R is the radius of the tissue (m).

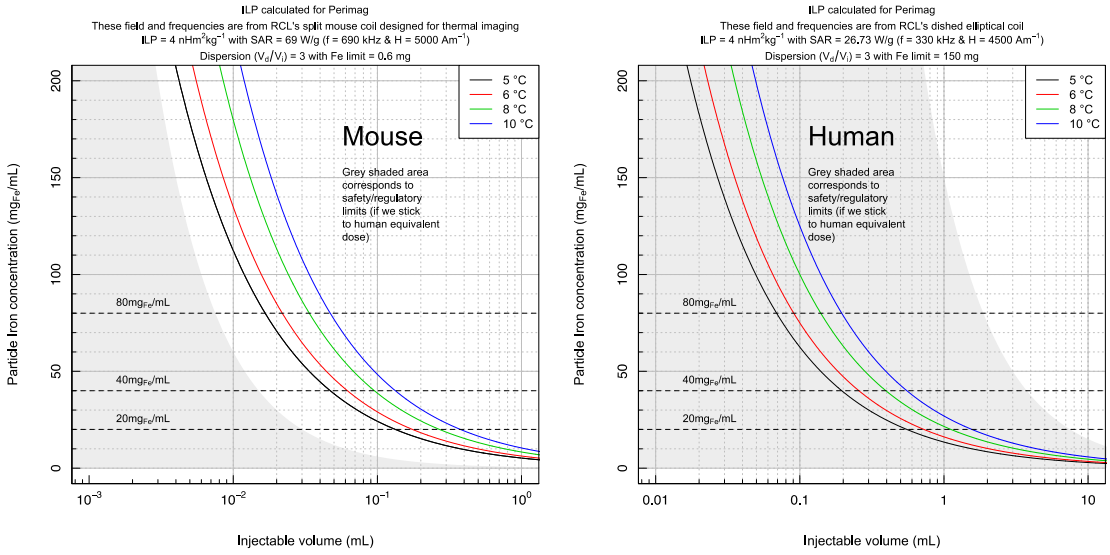

Figure 16: Bioheat models for mouse (left) and human (right) magnetic hyperthermia applications.

The limiting factor that prevents therapeutic temperatures in small animal models is the Fe toxicity limit (currently suggested as 0.6 mg for an average sized mouse). Whereas for the human model, the Fe limit is set at 150 mg and thus the required temperature rise can be estimated through the appropriate concentration and injectable volume. These Fe limits have been derived from the use of predicate materials such as Resovist and Feraheme, both of which are introduced into the body intravenously. The IFU for Resovist from Agis Commercial Agencies Ltd. 2002 state the following:

- *The maximum dose tested in humans, 0.08 ml Resovist (equivalent to 2.24 mg Fe) per kg body weight, was well tolerated.*

  Which indicates that intravenous doses up to 2.24 mg_{Fe}/kg (or 40 μmol_{Fe}/kg) have been tested in humans with no adverse effects. The recommended dose of Feraheme is an initial 510 mg dose followed by a second 510 mg dose 3 to 8 days later.

- *Administer Feraheme as an intravenous infusion in 50-200 mL 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP over at least 15 minutes.*

  Assuming 60 kg as an adult human's minimum weight, the 510 mg dose corresponds to 8.5 mg_{Fe}/kg however this dose is considered as a "3 day dose" which in effect is equivalent to a daily dose of 2.8 mg_{Fe}/kg.

  Other Fe-oxide predicate materials such as Sienna+ and NanoTherm are injected interstitially and in the case of NanoTherm offer much higher doses (35 mg_{Fe}/kg) based upon data in the literature (Johannsen et al., 2007). The rationale being that interstitial injection into the tumour would provide a higher level of safe Fe loading, yet the upper limit for circulatory Fe would be lower. Considering the worst case scenario whereby the injection is accidentally injected into the circulatory system, the upper limit for Fe to avoid toxic shock should be governed by intravenous limits and therefore for a 60kg human an Fe limit of 150mg was chosen. For the case of animal models, the Fe limit has been scaled according to a human equivalent dose based upon the conversion factors in Table 4.

Table 4. Conversion of Animal Doses to Human Equivalent Doses Based on Body Surface Area.

Species	To Convert Animal Dose in mg/kg to Dose in mg/m^2, Multiply by k_m	To Convert Animal Dose in mg/kg to HED[a] in mg/kg, Either:	
		Divide Animal Dose By	Multiply Animal Dose By
Human	37	---	---
Child (20 kg)[b]	25	---	---
Mouse	3	12.3	0.08
Hamster	5	7.4	0.13
Rat	6	6.2	0.16
Ferret	7	5.3	0.19
Guinea pig	8	4.6	0.22
Rabbit	12	3.1	0.32
Dog	20	1.8	0.54
Primates:			
Monkeys[c]	12	3.1	0.32
Marmoset	6	6.2	0.16
Squirrel monkey	7	5.3	0.19
Baboon	20	1.8	0.54
Micro-pig	27	1.4	0.73
Mini-pig	35	1.1	0.95

[a] Assumes 60 kg human. For species not listed or for weights outside the standard ranges, HED can be calculated from the following formula:

HED = animal dose in mg/kg x (animal weight in kg/human weight in kg)$^{0.33}$.

[b] This k_m value is provided for reference only since healthy children will rarely be volunteers for phase 1 trials.

[c] For example, cynomolgus, rhesus, and stumptail.

1.3.4 TOPASS

The major challenge of the DARTRIX project for TOPASS was testing specifically designed iron oxide nanoparticles for hyperthermia in a preclinical setting that allows evaluation of their risk-benefit profile in terms of their potential application in patients with glioblastoma. Various test protocols were realised in mice and rats that enabled selection of specific candidate nanoparticles for further development. Specifically, we found that the surface charge of iron oxide nanoparticles is critical for their risk profile after intravenous application. In this regard particles with a negative surface charge are more likely to be tolerated compared to particles with a positive surface charge. This finding is consistent with observations using nanoparticles with other coatings but comparable sizes (Lutz et al., 2006; Thunemann et al., 2006; Cartier et al., 2007; Kaufner et al., 2007).

In addition, we demonstrated the fate of iron oxide nanoparticles after implantation into the brain. The bulk of the material deposited inside the brain will stay there for a long time. Using *in vivo* imaging technologies such as MRI or PET the localization of nanoparticles could be followed over time inside the body of living animals. These protocols helped to implement the 3R concept (Refine, Reduce, Replace) for the DARTRIX project. Figures 17 and 18 demonstrate MR images of DARTRIX particles in the brain and liver of mice.

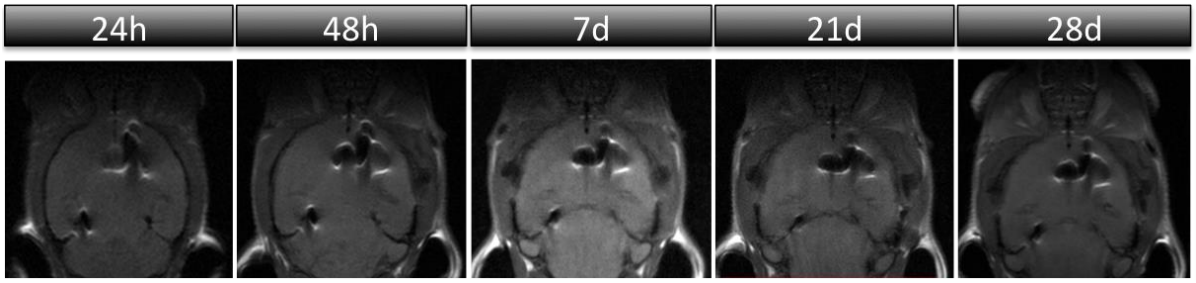

Figure 17: DARTRIX particles (perimag®-E69) in mice brain after deposition over time. The bulk of the particles are retained at the site of injection.

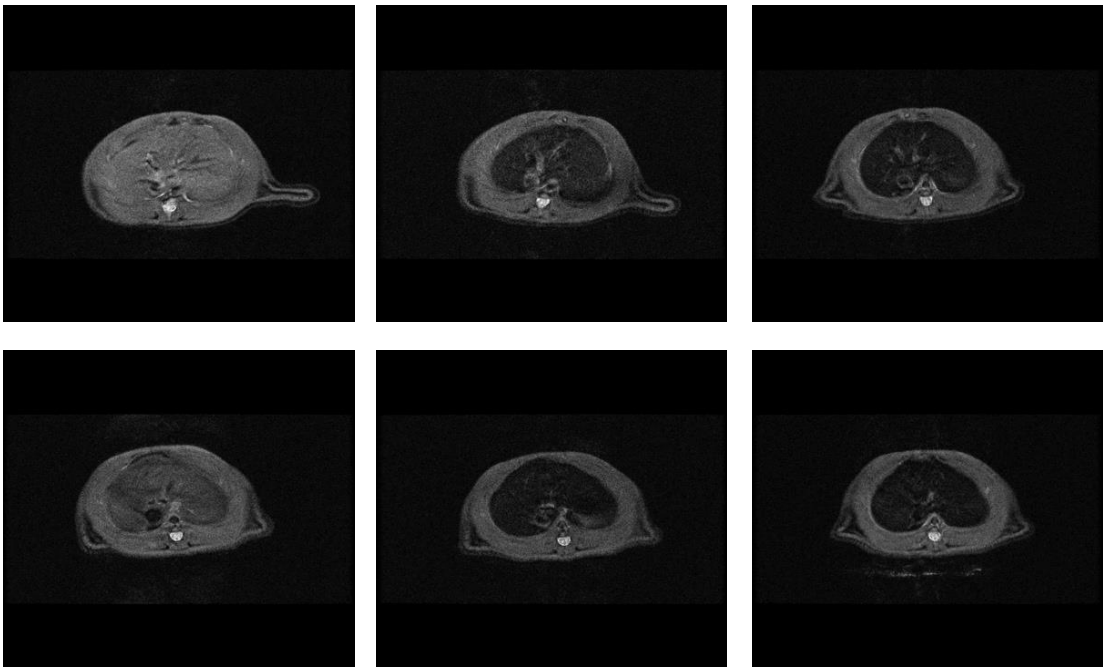

Figure 18: DARTRIX particles in the liver of living mice. The bulk of the particles are retained in the liver shortly after intravenous injection. Left: Baseline Scan. Centre: 10 minutes post IV injection. Right: 1 hour post injection.

TOPASS will publish the results of the *in vivo* studies using DARTRIX particles. The DARTRIX project helped to move the nanomedicine community to new frontiers by expanding the border of our knowledge concerning the behaviour of nanoparticles in living animals, specifically iron oxide nanoparticles.

1.3.5 UCL

UCL's major Scientific and Technical challenge was to develop a new bioprocess to manufacture DARPins that would be safe and effective as targeting agents for DARTRIX particles in the clinic. Initially we investigated manufacture in bacteria (*E. coli*) in accordance with published pre-clinical work on DARPins. However, after full evaluation we chose to use the yeast *P. pastoris* system as this allowed secretion of DARPin into culture broth, facilitating ease of obtaining pure product in a relatively simple process. Downstream processing of recombinant proteins produced by *P. pastoris* is easier than processing of recombinant proteins produced by *E. coli*, as only low concentrations of *P. pastoris* host cell proteins are released into the media. We worked closely with UZH in developing the process for E69 anti-EGFR DARPin and introduced a mutation to remove a potential breakdown site and improve yield of high integrity product. The process we developed was rapid and feasible to adapt to the rigors of manufacture to standards of Good Manufacturing Practice (GMP) in a quality controlled environment. This standard is a regulatory requirement for products used in patients.

1.3.5.1 Manufacture of GMP anti-EGFR DARPin

A 10L fermenter was used for process development and a 20L Clean in Place (CIP) machine for GMP. Figure 19 shows the complexity of fermentation apparatus required to provide a suitable controlled environment for the process. The high cell density of yeast culture is illustrated in the glass walled 10 Litre vessel on the left.

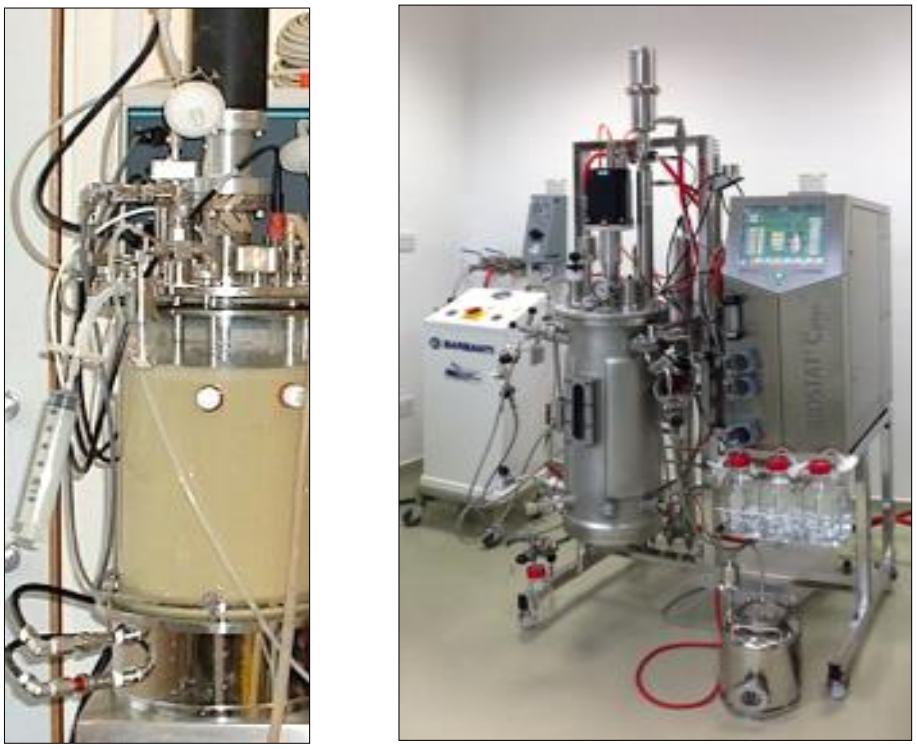

Figure 19: (Left) 10L Bioreactor for Process Development. (Right) 20L CIP Bioreactor in GMP Facility.

An outline of the steps for upstream processing (USP) and downstream processing (DSP) is shown in Figure 20; each step was controlled by robust standard operating procedures (SOPs) written for the project. The final product was pure with no evidence of aggregation as illustrated in Figure 21.

A

Cleaning in Place (CIP) of Bioreactor ▼	Day 1
Sterilisation in Place (SIP) of Bioreactor ▼	Day 2
Starting Primary Culture from Master Cell Bank ▼	
Starting Secondary Culture for Inoculation Bioreactor ▼	Day 3
Preparation / Addition Media ▼	
Addition of supplement Media and Bioreactor Setup ▼	Day 4
Inoculation of Bioreactor ▼	
Glycerol and Methanol Feeding ▼	
Bioreactor Harvest	

B

Concentrate & dialyze target protein ▼	Day 1
Size Exclusion Chromatography ▼	
Ion Exchange Chromatography ▼	
Desalting ▼	Day 2
CMPU API Fill	

Figure 20: Panel A and B, critical steps during the USP and DSP stage, respectively.

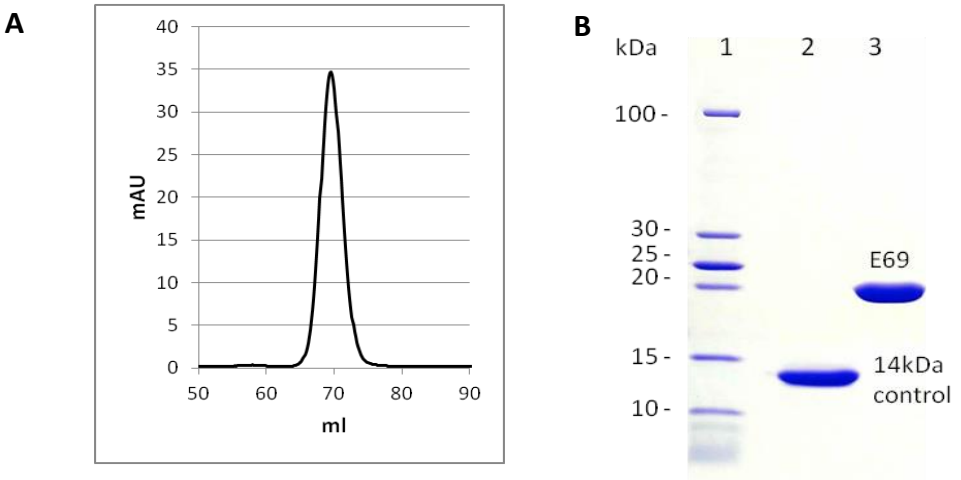

Figure 21: Aggregate analysis of E69: (**A)** Size exclusion profile; a sample of 1 ml was loaded onto a Superdex 75 column. **(B)** SDS-PAGE analysis of E69. A sample of E69 was applied to a 16% gel under reducing conditions. Lane 1: molecular weight marker; lane 2 14kDa control protein; lane 3, E69 from batch 2017#E69.

The final fill of E69 as an active pharmaceutical ingredient (API) was performed in a vertical laminar flow hood (VLFH) maintained in the GMP facility to a grade A standard (sterile environment). To ensure sterility and appropriate testing of the process the following SOPs were employed: use of biocides and alcohol to ensure a clean/sterile environment and use of special garments (see Figure 22). Settle plates were in use to monitor sterility of the environment; Sabouraud Dextrose Agar (SAB) to detect fungal and Tryptone Soya Agar (TSA) for bacterial contaminations; upon incubation at 22.5 and 32.5 ^{o}C for 3 days, these plates will show fungal or bacterial growth in case there is a breach of sterility in the hood. In addition, finger dabs on TSA and SAB plates are taken from the operator. Finally, 1ml aliquots were prepared in cryo-vials, labelled, racked and placed in a monitored ultra-low temperature freezer (-80^{o}C) (Figure 22). The final product was tested according to set release specification and each test was reviewed by the QC and QA Managers. The product met all release criteria regarding: concentration, fill volume, product size & purity, aggregate analysis, endotoxin, host cell protein, sterility, EGFR binding and mass spectrometry and was qualified with a certificate of analysis (CoA).

Figure 22: Preparation for final fill of E69 protein in the grade 'A' VLFH space (left) and Final fill vial rack for a GMP compliant batch.

1.3.5.2 DARTRIX Particle interactions with biological systems

In addition to overcoming challenges of GMP manufacture, UCL also developed *in vitro* and *in vivo* models to characterise interactions of DARTRIX particles with biological systems. This work included methods to reduce unwanted uptake of particles by the RES, as we initially published using near Infrared dyes (Abdollah et al., 2014) and later developed for radiolabelled imaging (Figure 23; manuscript in preparation).

The large range of *in vitro* cellular models developed by UCL included glioblastoma cells, paired isogenic cell lines (positive or negative for EGFR expression) neural stem cells and macrophages. Confocal Scanning Laser Microscopy (CLSM) was optimised to visualise particle internalisation into these cells and together with electron microscopy/mass spectrometry the models provided powerful tools to analyse cellular/particle interactions (Figure 24).

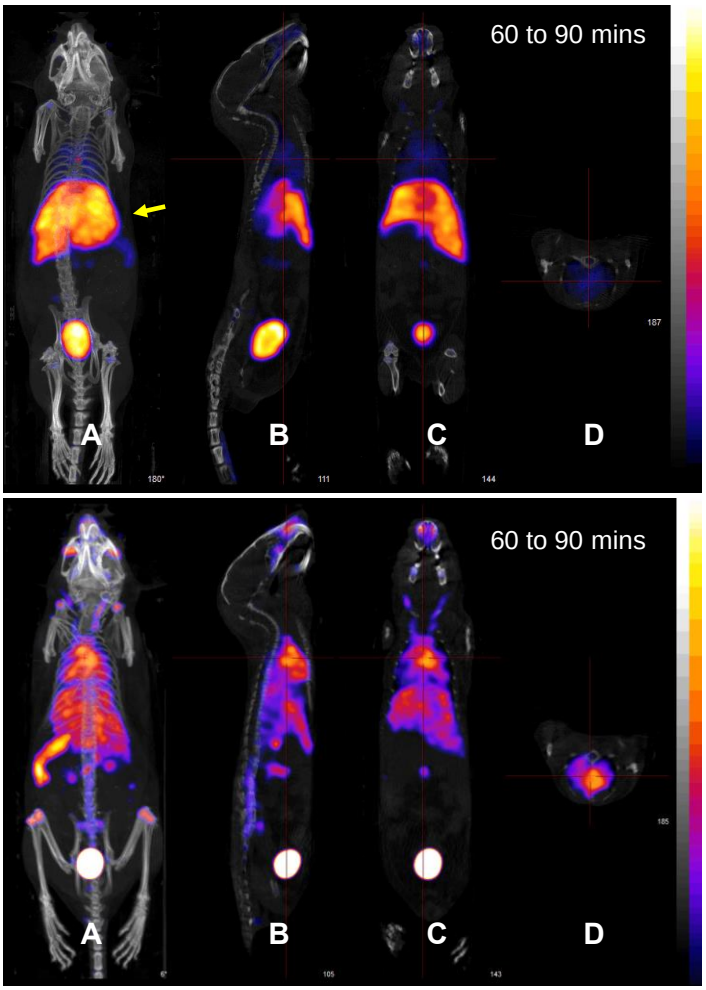

Figure 23: Single-photon emission computed tomography (SPECT) images of radiolabelled particles showing bio-distribution with or without treatment to block RES uptake. Top panel, (untreated) SPIONs taken up by liver (yellow arrow). Bottom panel, (Treated) SPIONs remain longer in circulation.

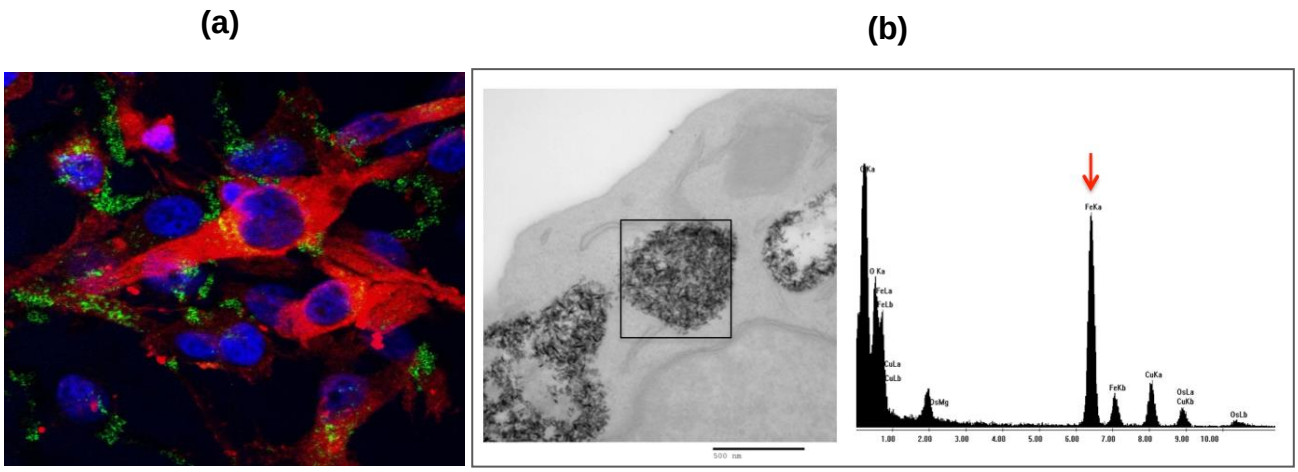

Figure 24: (a) CLSM image showing interaction of particles with human glioma (U-87 MG) cells *in vitro*. Cell membrane is stained red, SPIONs are shown in green while the nuclei are counterstained in blue. Z-stacks were performed to confirm internalisation. **(b)** TEM sections of macrophages showing electron dense aggregates within the cytoplasm (left). X-ray microanalysis used to confirm the presence of iron in the aggregates (right); an iron peak (red arrow) was observed in the vesicles containing the rod shaped aggregates.

To evaluate specificity of DARTRIX particles, UCL employed fluorescence-activated cell sorting (FACS). The most recent application of this method was to demonstrate EGFR-specific uptake of new DARTRIX particles that were functionalised with DARPins bearing PAS or XTEN long polar extensions (Figure 25).

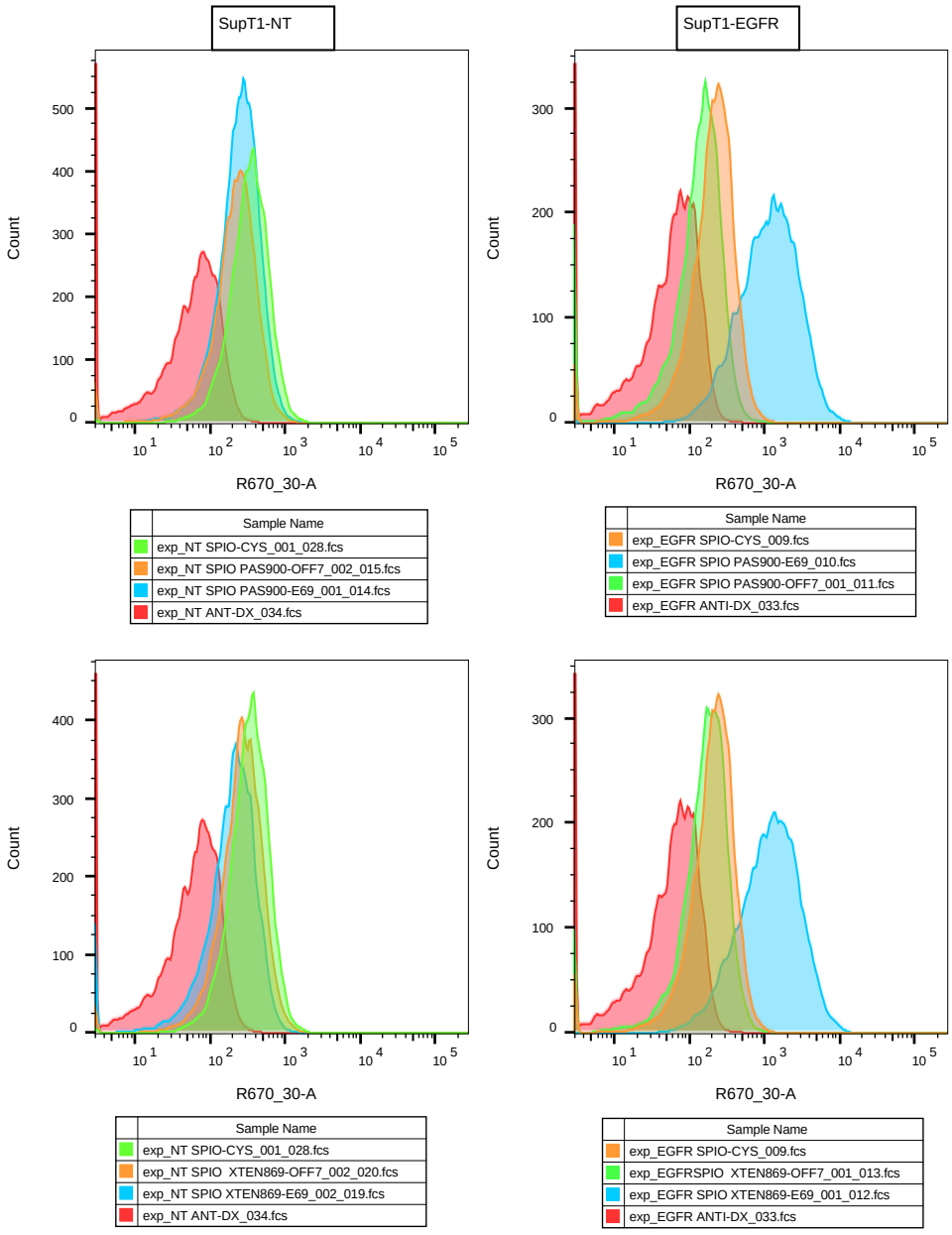

Figure 25: Cell binding of The PAS/XTEN-DARPin conjugated DARTRIX particles. EGFR negative cells (A and C) are shown in the left and EGFR positive cells (**B** and **D)** are shown on the right.

Anti-EGFR PAS-DARPin-conjugated DARTRIX particles (SPIO PAS900-E69 [**A** and **B**]) and anti-EGFR XTEN-DARPin-conjugated DARTRIX particles (SPIO XTEN869-E69 [**C** and **D**]), showed clear EGFR-specific cell binding to EGFR positive cells in comparison to the corresponding control (Off7) DARPin-conjugated DARTRIX particles (), the cysteine-labeled reference particles () and the background control (Anti-Dx).

1.4 The potential and the main dissemination activities and exploitation of results

1.4.1 UZH

1.4.1.1 Advantages displayed by the new materials by comparison with classical antibodies or Oligonucleotides

The strategy of the DARTRIX project contains a coupling step of the targeting agent to the magnetic nanoparticles. Furthermore, the nanoparticles need to be heated in the final treatment. These two requirements immediately show that highly stable, genetically modifiable binding proteins such as the DARPins have some key advantages. They can be genetically fused to very long unstructured regions, such that they have no steric hindrance to bind to cells. Furthermore, their high intrinsic stability make them resistant to heat-induced aggregation, a problem frequently encountered with many antibody fragments.

1.4.1.2 Clinical proof of concept and safety (lack of immunogenicity)

DARPins have been shown in clinical trials to not cause an immune response that would limit exposure, even after multiple injections over a prolonged period. This is due to the absence of aggregation (which would otherwise trigger a T-cell independent reaction) as well as the absence of T-cell epitopes in clinical constructs.

1.4.1.3 Scale-up and production methods

The scale-up of DARPins is very economic, and very robust against modifications of the molecules, such as additional cysteines or long unstructured extensions, as used in the present project.

1.4.2 RCL

The potential benefit to RCL lies with validation of a CE-ready medical device during first in man trials. It is also crucial that RCL can lay some fundamental ground rules for patient safety and tolerance to alternating magnetic fields. To date there is only one other company (MagForce, Germany) who have performed magnetic hyperthermia experiments, however the difference with RCL technology is the compactness of the device and the ability to apply the magnetic field with more focus to the region of interest. As far as RCL is aware there currently does not exist a CE Marked magnetic hyperthermia instrument for human use and based upon our progress through EMC tests as well as advice from our regulatory advisor we are confident that the equipment will be CE certified in the near future.

It is also worth noting that alongside development of the clinical system, RCL has gained insight into manufacturing characterisation and in vivo animal systems that have been used throughout the project. Pre-clinical data will play an important role in deciding on critical parameters for a clinical study such as field amplitude and exposure duration based upon magnetic nanoparticle dose. To this effect RCL has submitted a paper to the International Journal of Hyperthermia titled “Commentary on the clinical and preclinical dosage limits of interstitially administered magnetic fluids for therapeutic hyperthermia based on current practice and efficacy models”. Currently under review with a first

round of minor corrections, the paper aims to review and suggest suitable dose limits for magnetic nanoparticles under a range of pre-clinical and clinical conditions. Once again, RCL is unaware of any peer reviewed guidelines for magnetic hyperthermia and aims to disseminate results gained from the project.

1.4.3 MICROMOD

A very important initial result of the DARTRIX project for MICROMOD was the finding that perimag® particles provide high heating rates under the conditions of the MACH system from RCL. This makes the particles interesting for hyperthermia applications not only for the treatment of glioblastoma but also for other types of cancer in the future. The consistency of the heating properties of perimag® particles was clearly demonstrated. Within the DARTRIX project different surface modifications of perimag® were investigated regarding cell uptake and cytotoxicity. Perimag® with carboxylic acid groups on the surface were found to be non-toxic in *in vivo* mice studies up to a dose of 1.5 mg Fe per kg body weight. This information forms an essential framework to support our customers for the design of future medical applications.

The new generation of PAS- and XTEN DARPins from UZH was successfully conjugated to perimag® particles. A high specificity for target cells of the conjugates was demonstrated by *in vitro* cell uptake studies at UCL. The site-specific conjugation of perimag® to DARPins does not influence the biofunctionality of the DARPin molecules. This makes the perimag® particles a universal platform for the conjugation of different target molecules. These findings might strongly support the design of further investigations on glioblastoma therapy with hyperthermia.

MICROMOD has introduced perimag® as a standard catalog product into the market in January 2017. The advertisement for perimag® started at the 11th International Conference on the Scientific and Clinical Applications of Magnetic Carriers in Vancouver in May 2016 and was continued at the 7th International Workshop on Magnetic Particle Imaging IWMPI in Prague in March 2017. The distribution will also be supported by the product presentation of perimag® at the 34th Annual Society for Thermal Medicine Meeting in Cancun in May 2017.

The mentoring of MICROMOD's customers regarding customised conjugation of biomolecules on the surface of perimag® will be improved. The manufacturing of perimag® under clean room conditions will be offered at a high level of GMP compliance.

1.4.4 UCL

1.4.4.1 Clinical Impact

By developing a complete novel cGMP manufacturing system for DARPins using the yeast expression system, UCL's work during the DARTRIX project will have clear impact on future scale-up and production methods for DARPins and other recombinant proteins destined for the clinic. Both the upstream processing (USP) and downstream processing (DSP) protocols are designed to be readily scaled-up and are streamlined in ways to improve cost of goods. For example, reduced effort in removal of contaminants, no requirement for centrifugation, cost effective chemically defined media and no use of animal derived products.

UCL will also contribute substantially to potential clinical impact of the project by exploiting findings in the DARTRIX project to develop treatments that are led by the needs of brain cancer patients. There is an urgent need for an enhanced program of clinical trials in brain tumour patients and the

DARTRIX project will lead to a first in human clinical trial in glioblastoma patients. Currently, new therapies are first developed in other tumour types and only subsequently evaluated for use in brain cancer. Indeed, only 3% of brain tumour patients enter clinical trials (NCIN Routes to Diagnosis Report, published September 2012) and it is rare to have 'first in human' trials for brain cancer in the UK (Rampling et al. 2016). UCLs work, combined with other members of the DARTRIX consortium, will make strong contributions to the future treatment of brain cancer because information, knowledge and research outcomes from the DARTRIX project will underpin the clinical trials to take forward the new DARTRIX particles. These contributions include DARTRIX reported data on: favourable *in vitro* and *in vivo* heating potential in the MACH system, acceptable toxicity profile demonstrated in GLP studies, potential targetability of DARTRIX particles with a range of DARPins, stability and GMP compliance of DARTRIX particles. The strategic importance of this research into brain tumours is evident: in the UK alone approximately 9,000 people are diagnosed with primary brain tumour each year and there are almost 5,000 deaths. The majority of these deaths are attributed to a diagnosis of glioma. The current standard treatment following surgery in glioblastoma is 6 weeks of chemoradiotherapy followed by 6 months of temozolomide for those patients who have adequate performance status. For grade II and III glioma, there is marked improvement in overall survival with the addition of adjuvant systemic chemotherapy to radiotherapy (Buckner et al. 2016; van den Bent et al. 2013; Cairncross et al. 2013). However, the survival increase in glioblastoma is only modest with a median overall survival of 14.6 months (Stupp et al. 2005). There is no standard treatment for recurrent glioblastoma and options include best supportive care, nitrosureas or clinical trials. The median overall survival in recurrent glioblastoma is 5-10 months (Wick et al. 2010; Batchelor et al. 2013; Brown et al. 2016).

UCL will continue efforts to develop the DARTRIX concept for brain tumour patients before other patient groups. However, it is recognised that the DARTRIX project opens the gates to new treatments for other cancers and this will have a greater impact on public health. Indeed we have already opened negotiation to start UCL collaborations to take DARTRIX forward for patients with Melanoma and Head & Neck cancers. To date DARTRIX project work has secured funding from The British Council Russia Institutional Links (£145,951.00): Thermotherapy for the treatment of malignant brain tumours mediated by functionalised magnetic nanoparticles. The next stage will be to combine DARTRIX with immunotherapy investigating the role of therapeutic heating in reversing the hostile immune environment within a tumour. The rationale is strong: in the last decade, scientific and clinical research has demonstrated the ability of the immune system to control and potentially cure cancer and these ground breaking developments are driving a new era of therapeutic strategies for cancer treatment. We will build on our recent safety observations in the clinical use of immune checkpoint inhibitors in glioblastoma (Carter et al 2016). A further grant has been submitted to the "The Brain Tumour Charity: Quest For Cures" application call under the title of "Harnessing the Immune System in Glioma" in part, to take forward the work of the DARTRIX project. This grant application has been led by UCL.

1.4.4.2 Educational Impact

The DARTRIX project has been highlighted at two significant educational events with potential to influence future thinking; at "Antibodies: An evolving force in cancer treatment" (11 March 2015), the DARTRIX project was discussed in the context of glioblastoma treatment and future directions for research. The meeting was organised by UCL DARTRIX partners for the Oncology Section at the Royal Society of Medicine. It explored the current and future developments in the use of antibodies and DARPins for the diagnosis and treatment of cancer. There were approximately 70 attendees from diverse backgrounds, Medicine (Students to Consultants/Professors), Scientists (PhD students, Postdoctoral Fellows, Professors), Charity employees and Patent attorneys. A second Educational

DARTRIX presentation was given as part of a Royal Society of Medicine Acute Oncology Education Day (15 January 2016).

The DARTRIX project funded a clinical fellow who has used their time to develop their doctoral research. In addition the fellow has been educated in the management of glioma patients and in clinical trial methodology. The fellow has developed a keen interest in glioma treatment and is ideally placed to take a leadership role in the future development of treatments for brain cancer patients.

1.4.5 EUROPEAN DIMENSION

Tumours arising in the central nervous system (CNS) make a significant impact on the health of the citizens of the European Union with approximately 27,700 new cases diagnosed each year (Crocetti et al. 2012). The DARTRIX project aimed to develop a new method of treatment for these cancers. By synergistic collaboration throughout the project, the consortium demonstrated complete success in bringing together resources that are not available in any single country. Thus addressing the recognised problem that cancer research is mainly undertaken at national level and tends to be considerably fragmented and diverse across the EU. During the DARTRIX project, DARPins were designed by UZH with a unique c-terminal cysteine for site specific attachment. UCL developed GMP-compliant methods for manufacturing the DARPins. MICROMOD generated a new range of DARTRIX particles, with surface chemistries tailored for attachment of DARPins. UCL and RCL developed methods for size fractionation of particles and determined the heating properties and UCL established methods to evaluate in vitro cell uptake and in vivo RES uptake of the DARTRIX particles. The consortium brought these skills together to achieve their ambitious goals. There is no single entity that has the academic, technical, clinical and commercial know-how to achieve this alone. Furthermore, private investors would be extremely unlikely support venture capital investment at this early stage although the potential benefits for participants are very high.

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1.4.6. Main Dissemination Activities

1.4.6.1 Publications

D.O.I.	Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Open access is/will be provided to this publication
10.1039/C4FD00114A	Prolonging the circulatory retention of SPIONs using dextran sulfate: in vivo tracking achieved by functionalisation with near-infrared dyes	Maha R. A. Abdollah , Tammy Kalber , Berend Tolner , Paul Southern , Joseph C. Bear , Mathew Robson , R. Barbara Pedley , Ivan P. Parkin , Quentin A. Pankhurst , Paul Mulholland , Kerry Chester	Faraday Discussions	175	Royal Society of Chemistry	United Kingdom	01/01/2014	41-58	Yes
http://dx.doi.org/10.1016/j.clon.2016.04.042	Ipilimumab and Bevacizumab in Glioblastoma	T. Carter , H. Shaw , D. Cohn-Brown , K. Chester , P. Mulholland	Clinical Oncology	Vol. 28/Issue 10	W.B. Saunders Ltd	United Kingdom	01/10/2016	622-626	Yes
10.2217/imt.16.11	Antibody-targeted nanoparticles for cancer treatment	Thomas Carter , Paul Mulholland , Kerry Chester	Immunotherapy	Vol. 8/Issue 8	Future Medicine Ltd.	United Kingdom	01/07/2016	941-958	Yes
-	Modifying the In Vivo Biodistribution and Pharmacokinetics of Dextran-Coated Iron Oxide Nanoparticles	Maha R. A. Abdollah, Berend Tolner, Julia Baguña Torres, Mathew Robson, R. Barbara Pedley, Paul Mulholland, Rafael T. M. de Rosales and Kerry Chester			ACS Nano	UCL	Suggested revisions in progress		Yes
-	Optimizing the heating potential of superparamagnetic iron oxide nanoparticles via fractionation with size-exclusion chromatography	Maha R.A Abdollah, Berend Tolner, Fang-Yu Lin, Paul Southern, Paul Mulholland, Quentin A. Pankhurst, and Kerry Chester			Nano Letters	UCL	Manuscript in preparation		Yes

D.O.I.	Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Open access is/will be provided to this publication
-	Superparamagnetic Iron-Oxide Nanoparticles (SPIONs) for Magnetic Hyperthermia; In-vivo SPION fate and tumour microenvironment responses	Thomas Carter, Matthew Ellis, Giulia Agliardi, Fang-Yu Lin, Clare Jones, Angela Richard-Londt, Mathew Robson, R. Barbara Pedley, Tammy Kalber, Quentin Pankhurst, Rafael T. M. de Rosales, Paul Mulholland, Sebastian Brandner, Kerry Chester			ACS Nano	UCL	Manuscript in preparation		Yes
-	MICROMOD will publish the results of the successful DARPin conjugation to perimag® particles together with UCL and UZH. Further publication of toxicity data and magneto-physical characterization is envisaged.	TBC			TBC	MICROMOD			Yes
-	Commentary on the clinical and preclinical dosage limits of interstitially administered magnetic fluids for therapeutic hyperthermia based on current practice and efficacy models.	RCL and associates			International Journal of Hyperthermia	RCL	Submitted		Yes

1.4.6.2 Conferences and presentations

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	NANOPET PHARMA GMBH	MoBi 2012 (Molecular Imaging 2012; 17.09.-19.09.2012)	17/09/2012	Erlangen, Germany	Scientific community (higher education, Research) - Industry	Germany
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Inter-Particle Interactions in the Static and Dynamic Magnetic Properties of Ferucarbotran Colloids	22/05/2012	Minneapolis, USA	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Advances in Magnetic Hyperthermia	05/07/2012	Toyohashi University, Japan	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Biomagnetic Applications for Magnetic Nanoparticle	09/04/2013	University of York, UK	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Biomagnetic Applications for Magnetic Nanoparticles	26/04/2013	Technical University of Denmark (DTU)	Scientific community (higher education, Research)	United Kingdom

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Molekulare Bildgebung	17/09/2012	Erlangen, Germany	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Developing Antibody - Targeted Nanoparticles for Cancer Therapy	25/06/2012	Mykonos, Greece	Scientific community (higher education, Research)	United Kingdom
Flyers	NOVAMEN SAS	DARTRIX Flyer	16/04/2013	DARTRIX Website	Scientific community (higher education, Research)	France
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	ACS 245th National Meeting	08/04/2013	New Orleans	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Which tools for tumor targeting? Workshop at the Institute de Recherche pour la Santé	18/06/2013	Nantes, France	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	38th FEBS Congress 2013"Mechanisms in Biology"	10/07/2013	St. Petersburg, Russia	Scientific community (higher education, Research)	Switzerland

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	MPI Frankfurt Departments-Symposium "Signalling across membranes"	15/07/2013	Tegernsee, Germany	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	10th International PhD Student Symposium "Horizons in Molecular Biology"	11/09/2013	Göttingen, Germany	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Canada Gairdner International Awards Symposium	25/10/2013	Toronto, Canada	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Antibody-Targeted Nanoparticles for Cancer Treatment	28/01/2013	Vancouver, Canada	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Designing receptor binding proteins with highly potent biological function	05/11/2013	PEGS Europe, Lisbon, Portugal	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineering protein ligands for very powerful biological response	11/11/2013	9th European Antibody Congress 2013, Geneva, Switzerland	Scientific community (higher education, Research) - Industry	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Combinatorial and evolutionary engineering: the binders and the targets	06/12/2013	2013 Beckman Symposium, City of Hope, CA, USA	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineering powerful ligands for ErbB receptors	04/02/2014	Workshop 'Frontiers in Biology and Medicine', Bangalore, India	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineering powerful ligands for ErbB receptors	25/03/2014	Intl. Workshop: New Approaches in Drug Design & Discovery, Rauischholzhausen, Germany	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Combinatorial and evolutionary protein engineering	26/06/2014	6th Biocenter Basel PhD Symposium. Engelberg, Switzerland	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineering HER2-targeting ligands for powerful downstream responses	24/07/2014	2014 FASEB Science Research Conference, Snowmass, CO, USA	Scientific community (higher education, Research) - Industry	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Cell-specific uptake of proteins to the cytoplasm through engineered import mechanisms	04/09/2014	Roche Nature Biotechnology Symposium, Buonas, Switzerland	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Fluorescent DARPins as sensors and detectors	25/09/2014	Labelling & Nanoscopy, German Cancer Research Institute, Heidelberg, Germany	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Can intracellular proteins be targeted?	08/12/2014	IBC's 25th Annual Antibody Eng. & Therapeutics Conference, Huntington Beach, CA, USA	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	The potential of DARPins for cancer treatment	11/03/2015	Royal Society of Medicine, London, U.K.,	Scientific community (higher education, Research)	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Modular recognition system for the sequence-specific binding of peptides: design and evolution of Armadillo Repeat Proteins	31/03/2015	Biochemical Society - Repetitive, Non-Globular Proteins: Nature to Nanotechnology, York, U.K.	Scientific community (higher education, Research)	International
Oral presentation to a wider public	NANOPET PHARMA GMBH	BIONNALE - Biotech Conference	06/05/2013	Germany, Berlin	Scientific community (higher education, Research) - Industry	Germany
Posters	UNIVERSITY COLLEGE LONDON	Developing Superparamagnetic Iron Oxide Nanoparticles (SPIONs) as Cancer Nanomedicines.	18/03/2015	London, UK	Scientific community (higher education, Research)	United Kingdom
Posters	UNIVERSITY COLLEGE LONDON	Developing Superparamagnetic Iron Oxide Nanoparticles (SPIONs) as Cancer Nanomedicines.	03/11/2013	BT Convention Centre Liverpool, UK.	Scientific community (higher education, Research)	United Kingdom
Posters	UNIVERSITY COLLEGE LONDON	Superparamagnetic iron oxide nanoparticles (spions) for the hyperthermic treatment of malignant melanoma.	06/11/2015	BT Convention Centre Liverpool, UK.	Scientific community (higher education, Research)	United Kingdom

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Posters	UNIVERSITY COLLEGE LONDON	Estimating the Power Absorption of Iron Oxide Nanoparticles from AC Susceptiblity Measurements	05/06/2014	Dresden, Germany	Scientific community (higher education, Research) - Industry	UE
Posters	UNIVERSITY COLLEGE LONDON	Targeted Magnetic Hyperthermia for Treatment of Glioblastoma	18/03/2015	University College London (UCL). London, UK	Scientific community (higher education, Research) - Industry - Civil society	United Kingdom
Posters	UNIVERSITY COLLEGE LONDON	Superparamagnetic iron oxide nanoparticles (spions) for the hyperthermic treatment of malignant melanoma.	03/11/2013	BT Convention Centre Liverpool, UK.	Scientific community (higher education, Research) - Civil society	United Kingdom
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Prolonging the circulatory retention of SPIONs using dextran sulfate: in vivo tracking achieved by functionalisation with near-infrared dyes.	05/09/2014	Bristol, UK	Scientific community (higher education, Research)	United Kingdom
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	New engineering and application perspectives of scaffold proteins	18/05/2015	IBC's Next Generation Protein Therapeutics, San Francisco, CA, USA	Scientific community (higher education, Research)	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Combinatorial and evolutionary protein engineering	28/05/2015	Goethe University Biochemistry II, Seminar Series, Frankfurt, Germany	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering: The key to empowering therapeutic proteins in novel ways	17/06/2015	Empowered Antibodies Congress, Barcelona, Spain	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineered and evolved binding proteins to influence cell signaling	15/09/2015	University of Fribourg, Biology/Biochemistry, Fribourg, Switzerland	Scientific community (higher education, Research)	Switzerland
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Combinatorial and evolutionary protein engineering	06/10/2015	University of Bristol, School of Cellular and Molecular Medicine, Bristol, UK	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering for new modes of actions and new targets	02/11/2015	PEGS Europe 'Protein & Antibody Engineering Summit', Lisbon, Portugal	Scientific community (higher education, Research)	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering approaches to new biologicals	07/03/2016	Keystone Symposia on Antibodies as Drugs, Whistler, BC, Canada	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering approaches to new biologicals	04/07/2016	4th Antibody Industrial Symposium, Montpellier, France	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	IBiomedical Applicatuions of Magnetic Nanoparticles	15/09/2015	International Conference on the Applications of the Mossbauer Effect, Hamburg, GERMANY	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	UK-Japan Bioengineering Workshop	04/12/2015	Japanese Embassy, London, UK	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Translational R&D in Nanoparticulate Medical Devices	04/12/2016	Nanostructures for Medical Applications, York, UK	Scientific community (higher education, Research) - Industry	Europe

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	International Conference on Hyperfine Interactions and their Applications	08/07/2016	Leubven, BELGIUM	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Biomedical Applications for Magnetic Nanoparticles	26/05/2016	Sheffield University Seminar Series, UK	Scientific community (higher education, Research)	UK
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	Remote Monitoring of Magnetic Particle Temperature During Hyperthermia: the Ideal Dose-Response Metric?	04/05/2016	11th International Conference on the Scientific and Clinical Applications of MagnetVancouver, Canada	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	RESONANT CIRCUITS LIMITED	On the reliable measurement of specific absorption rates and intrinsic loss parameters in magnetic hyperthermia materials	28/10/2016	COST Action: RADIOMAG - Magnetic hyperthermia, Limasssol, Cyprus	Scientific community (higher education, Research) - Industry	Europe
Posters	RESONANT CIRCUITS LIMITED	Field and frequency dependence of the SAR/ILP value in magnetic hyperthermia using magnetic multi- and single core particles	05/06/2016	International conference on magnetism, Barcelona, Spain	Scientific community (higher education, Research) - Industry	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Posters	UNIVERSITY COLLEGE LONDON	Evaluating the Effectiveness of RES Uptake Blockers using Radiolabeled Nanoparticles and SPECT Imaging	09/11/2015	2nd Preclinical Nuclear Imaging Symposium -UK	Scientific community (higher education, Research)	UK
Posters	UNIVERSITY COLLEGE LONDON	Evaluating the Effectiveness of RES Uptake Blockers using Radiolabeled Nanoparticles and SPECT Imaging	18/10/2016	UCL Cancer Institute open day, UK	Scientific community (higher education, Research)	UK, EU
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Modifying the Pharmacokinetics of Iron Oxide Nanoparticles to Enhance Their Therapeutic Potential	18/10/2016	UCL Research in progress seminar, UK	Scientific community (higher education, Research)	UK, EU
Posters	UNIVERSITY COLLEGE LONDON	Targeted Magnetic Hyperthermia for Treatment of Glioblastoma	13/03/2016	UCL Cancer Institute Student Poster Day	Scientific community (higher education, Research)	UK, EU
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Glioblastoma: Are we seeing the first cures?	20/05/2016	UCLH Grand Round, UK	Scientific community (higher education, Research)	UK
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Keynote Presentation Superparamagnetic Iron Oxide Particles (SPION) for Cancer Treatment	02/05/2016	EMRS Symposium Multifunctional nanostructures for diagnostic and therapeutic of diseases-Lille FR	Scientific community (higher education, Research)	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Using protein engineering to enable drug development	23/09/2016	Gilead Sciences, Inc. Seminar Series, Foster City, CA, USA	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Engineering approaches for targeting proteins in the cytosol	25/10/2016	Sanofi SA Workshop, Paris, France	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering for new modes of action	03/11/2016	PEGS Europe, Lisbon, Portugal	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	Protein engineering for inducing cell-selective apoptosis	14/11/2016	BCMP Symposium, Harvard Medical School, Boston, MA, USA	Scientific community (higher education, Research) - Industry	International
Oral presentation to a scientific event	UNIVERSITAET ZUERICH	New therapies from protein engineering and the study of cell signaling	25/01/2017	Keystone Symposia, Snowbird, Utah, USA	Scientific community (higher education, Research) - Industry	International

Type of activities	Main leader	Title	Date	Place	Type of audience	Countries addressed
Oral presentation to a scientific event	MICROMOD PARTIKELTECHNOLOGIE GMBH	11th International Conference on the Scientific and Clinical Applications of Magnetic Carriers	01/05/2016	Vancouver, Canada	Scientific community (higher education, Research)	International
Forthcoming presentations						
Oral presentation to a scientific event	MICROMOD PARTIKELTECHNOLOGIE GMBH	7th International Workshop on Magnetic Particle Imaging IWMPI	01/03/2017	Prague	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	MICROMOD PARTIKELTECHNOLOGIE GMBH	34th Annual Society for Thermal Medicine Meeting	01/05/2017	Cancun, Mexico	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Immunotherapy for Glioma Patients	12/06/2017	Advances in the Applications of Monoclonal Antibodies in Clinical Oncology and Symposium on Neuro-Oncology; Cyprus,	Scientific community (higher education, Research)	International
Oral presentation to a scientific event	UNIVERSITY COLLEGE LONDON	Nanomedicine for Hyperthermia in Glioblastoma	12/06/2017	Advances in the Applications of Monoclonal Antibodies in Clinical Oncology and Symposium on Neuro-Oncology; Cyprus,	Scientific community (higher education, Research)	International

1.4.6.3 Exploitation of results

As outlined in the initial description of work and reported throughout the project, the consortium made significant progress towards a future DARPin Targeted Therapy and has developed valuable IP. Through close monitoring and evaluating the progressing DARTRIX project results the consortium members were able to extend and strengthen their IPR portfolio for future exploitation. The handbook of Foreground IP has been updated accordingly to represent all IP generated throughout the project by all consortium members. While the dissemination of the "longer circulatory time for SPIONs through a clinical agent" and "fractionation of ferucarbotran" are being prepared through publications, UCLB is currently finishing the value assessment of potential patent filings.

IP generated	Owner	Foreseen use	Created	Type of IP
Modified DARPins	UZH	Modified and improved DARPin for the use in DARPIN targeted Therapy in Glioblastoma	2013	Know-how
Perimag® characterisation	MICROMOD	The generated surface modification, compatibility with RCL MACH system, site-specific conjugation of perimag® to DARPins proofs perimag® particles as a universal platform for the conjugation of different target molecule and forms an essential framework for future customised medical applications.	2016	Know-how
Voltage source driver for a parallel resonant magnetic field generator	RCL	MACH System (Medical Device)	2015	Patent application (US20160352329A1)
Temperature Measurement System And Method	RCL	MACH System (Medical Device)	2014	Patent application (WO2014128495)
Coil design for a more "focussed" application of the magnetic field	RCL	Improved clinical device. Potentially avoiding/limiting exposure of magnetic field to areas of the head	2015	Know-how
Manufacturing characterisation and pre-clinical data	RCL	Data which will play an important role in defining critical parameters for a clinical study such as field amplitude and exposure duration based upon magnetic nanoparticle dose	Throughout DARTRIX project	Know-how
The rapid circulatory clearance of ferucarbotran has been moderated by co-administration of a clinically compatible agent that appears to block RES uptake. This has been demonstrated by in vivo imaging with radiolabelled ferucarbotran	UCL	Longer circulatory time for SPIONs in vivo, using a clinically applicable agent	2015	Know-how and potential patent application under review
Fractionation of ferucarbotran to obtain a more homogeneous sub-population with improved heating properties.	UCL/RCL	Improved clinical product	2013	Know-how and potential patent application under review
A bioprocessing method for generating GMP DARPins using the Pichia pastoris expression system	UCL/UZH	Production of DARPins for clinical trials	2015	Know-how

While the DARPin Targeted Therapy still requires further development, pre-clinical and clinical valuation, some of the generated IP is already being commercially exploited through individual consortium members. As outlined in previous sections, the DARTRIX collaboration has helped MICROMOD to generate crucial data for perimag® nanoparticles which are now not only a standard catalogue product but are also meant to be used in other customised medical applications outside of DARTRIX. While having extended the patent portfolio around the proprietary MACH technology, RCL has also made a significant step towards CE marking of the system. A current market review has also confirmed that there is currently no other system available which is able to deliver a locally targeted magnetic field like the RCL system. While completing EMC tests and other CE marking activities RCL will also start to commercially exploit and develop the MACH system.

While MICROMOD and RCL are already, or in due course, in a position to exploit some results of the DARTRIX project the consortium members are continuing to progress the technologies towards a personalised glioblastoma thermotherapy. As such the consortium members, led by UCL, are in the preparation of a potential first-in-man clinical trial which would not only deliver the first glioblastoma thermotherapy but also would further strengthen and validate the foreground IP generated during the DARTRIX project. Said follow-on research, which is necessarily to bring the technology into the clinic to benefit glioblastoma patients, will also incorporate and benefit from know-how generated i.e. use of specific size fractions, valuable pre-clinical data, potential use of clinical agents to increase SPION circulation time. That said, until the first thermotherapy in glioblastoma is delivered and the subsequent up take in health care systems is still several years away but the consortium members are continuing to work together to ensure that further down the line said exploitation is successfully managed.

1.4.6.4 Address of the project public website and relevant contact details

http://www.dartrix.eu/

Dartrix