
1. Publishable summary

The ENCITE project

The project **European Network for Cell Imaging and Tracking Expertise (ENCITE)** comprises **five main areas of work**, namely novel imaging technologies, novel imaging reporter probes, novel tools for cell labelling, pre-clinical validation, and translation towards clinical application. The objectives are to develop new imaging methods to improve the tracking of labelled cells, procedures to cross-validate each individual approach and to develop new contrast agents to improve the sensitivity and specificity of cellular labelling.

Mission

To develop and test new MR and optical imaging methods and biomarkers to get a more comprehensive picture of cell fate and the reaction of the immune system and to ultimately improve and further develop cell therapy for the benefit of the European patient.

Summary of project objectives

In order to be able to address the extremely wide variety of cell therapies, the objectives of ENCITE have been divided into generic and disease-oriented objectives.

The generic project objectives include the development of new imaging methods to improve the spatio-temporal tracking of labelled cells, the generation of dual- and multimodality imaging procedures, and the generation of procedures that will improve the sensitivity and specificity of cellular labelling. For the generation of molecular and cellular imaging reporters, the potential of combining molecular biology with multimodal imaging techniques is being explored, and novel cell and animal reporter systems are being generated that will enable detection of the location and function of individual cells and small cell subsets within the target organ. Appropriate cellular labelling techniques that do not interfere with cellular functions and therapeutic efficacy are being established and new methods for quantitative assessment are being developed that will generate reliable biomarkers of the cell fate and therapeutic effects. The overall aim is to establish and validate cell homing for therapeutic delivery to target organs. Legal and ethical issues associated with the use of new agents and methodologies will be properly addressed.

Regarding disease-specific project objectives, the project aims at establishing the clinical potential of novel imaging approaches in preclinical studies related to neurological, cardiovascular and musculoskeletal diseases, as well as to cancer and diabetes. One of the major project objectives relates to the improvement of existing cell-based therapies in the treatment of cancer.

Description of the work performed in the first year

Within the work programme of novel imaging technologies significant progress has been made in the **testing and development of novel methods for cell tracking and the evaluation of new contrast agents**, in addition to the optimisation of known concepts. Work focused on methods for improved detection of iron oxide particles (USPIOS, VSPIOS, SPIOS, MPIOS...), as these are the most commonly used cell labelling agents within the consortium. Methodological developments covered both negative and positive contrast techniques as well as ongoing work on techniques for fluorinated (¹⁹F) labels and probes based on chemical exchange saturation transfer such as CEST, ParaCEST and LipoCEST. Considerable effort was also put into the **characterisation and development of new imaging biomarkers to characterise cell fate and development**. Significant inroads were made into the field of image registration and combination of multiple modalities available for preclinical evaluation, including bioluminescence, MR imaging and optical imaging tomography. An articulated atlas for image registration of follow-up studies was produced and will be deployed for multi-modal image registration later in the project.

The project work on novel imaging reporter probes comprised a range of activities on major classes of MRI contrast agents. Concerning iron-oxide based agents, work focused on developing methods for

improved control of size and surface characteristics. Paramagnetic Gd(III) complexes, characterised by high relaxivities were developed. It has been found that an efficient labelling of cells bearing CD44 receptors can be achieved using cationic liposomes covered by hyaluronic acid. Good progress has been made in the field of **responsive agents** by developing a probe whose relaxivity reports about the activity of beta-galactosidase, an enzyme largely used as reporter of gene expression. Upon cleavage of the exposed sugar moiety, the probe polymerizes with a concomitant increase of the observed relaxivity. Interesting results were obtained with an MRI paramagnetic probe able to target thiol-containing moieties. This approach appears particularly useful in the development of a cell labelling procedure based on the targeting of exofacial protein thiols.

Significant progress was made towards the development of novel tools for cell labelling. Cell labelling using exogenously administered, clinically approved contrast media that are internalised in perinuclear endosomes resulted in quenched relaxivity. Photodynamic therapy of the labelled cells caused a marked increase in the relaxivity attributable to the endosomal escape of the complex. Alternatively, endosomal escape using specific peptides has been evaluated. The **efficacy of cell labelling with iron oxide particles and with Gd-DTPA** has been studied in different types of cells. Two clinically approved iron-oxide-based contrast agents were evaluated for their suitability as intra-cellular probes for clinically applicable in vivo cell tracking of bone marrow derived stem cells and chondrocytes. In addition, the feasibility of using Gd-DTPA as an intra-cellular probe by means of incorporation into lipid-based nanoparticles was tested using mainly mesenchymal stem cells.

The **development of reporter genes** included the production of the Adenovirus TK reporter and a tumour cell line expressing ferritin along with luciferase. Differentiation of murine embryonic stem cells to generate neurons and fibroblast activation has been followed by optical imaging and MRI. In addition, a new approach is being developed for imaging cell death (apoptosis). After successful in vitro studies, in vivo analyses are now being performed. The detection of cell proliferation or apoptosis has also been evaluated through the use of quantitative mapping strategies and multi-sequence analysis techniques.

Pre clinical validation for the various disease areas has started. In the field of **neurological diseases** transgenic mice which express luciferase under DCX (doublecortin) control were generated. Pilot studies indicated that, with these transgenic mice, neurogenesis can be monitored in vivo. To allow in vivo monitoring of angiogenesis, the generation of transgenic mice that express a VE-cadherin-ferritin construct has been started. Novel CEST probes were generated that will allow simultaneous imaging of two types of cells. In the area of **cardiovascular diseases**, studies to define detection limits for iron-oxide-based probes and Gd-based probes using various acquisition sequences and protocols have been started using phantom preparations of cells labelled with either of the above probes. Furthermore, various MR acquisition strategies based on 3D-triggered, 3D-untriggered and 2D-triggered sequences were optimised and assessed in vivo in rodents. Longitudinal in vivo data sets from rodent hearts were generated to evaluate various imaging tools to assess cell migration and myocardial function. Work related to **musculoskeletal diseases** focused on the use of MRI for monitoring intervertebral disc ablation. Furthermore, CEST agents were implemented to measure glucose-aminoglycan (GAG) in the intervertebral disc. As for **diabetes**, novel dual modality probes suitable for cell tracking by both MRI and optical imaging were evaluated and a method for semi-automated detection and quantification of pancreatic islet survival was developed and evaluated. For **dendritic cell therapy** studies included the optimisation and implementation of protocols for labelling dendritic cells (DCs) with radioactive indium-111. Iron-oxide particles (SPIO) were used for labelling and imaging of DCs, yielding images with sharp anatomical detail and allowing for immunohistochemical validation. As far as **T-cell therapy** is concerned, cytotoxic T-lymphocytes recognizing Ovalbumin peptide with a large potential for killing OVA-peptide-expressing target cells in vitro were generated. Work related to **monitoring the dynamics of tumour and tumour microenvironment** focused on the development and evaluation of various optical imaging probes for the in vivo detection of apoptosis using multi-photon microscopy as well as the development of tools for in vivo imaging of recruitment and effects of tumour stroma fibroblasts.

Translation towards clinical applications was started for the fields of cancer and diabetes. **Cancer**-related work focused on culturing dendritic cells, designing the experiments, processing samples, doing flow cytometry, and evaluating the purity of the samples. A phase I/II trial (DERMA-ER-DC 06) has been conducted with sequential adaptive design in advanced stage IV melanoma patients using autologous dendritic cells and specific RNA as source for antigen loading. With respect to **diabetes**, a pilot study was carried out among patients with transplanted labelled pancreatic islets. A study of

steatosis has started to obtain a basic set of fat concentrations for the application of the method in patients with transplanted pancreatic islets. A pilot study was successfully performed using SSFP sequence to obtain positive contrast of iron labelled pancreatic islets.

Description of the main results achieved in the first year

In the field of novel imaging technologies, **MR imaging methods for cell tracking based on iron oxides** were successfully implemented, and clinical methodologies for preclinical evaluation of novel drugs were adapted and optimised. Whereas for negative contrast techniques, gradient echo imaging has turned out to cover the requirements of most applications very nicely, the development of positive contrast techniques still requires further work. Techniques have been developed based on un-balance gradient echo techniques, but also using modifications of turboCraze as well as ultrashort TE (UTE)-sequences. The **image postprocessing** group generated 3D and 4D datasets from the brain and the heart recorded under variable conditions using different MRI contrast and spatial resolutions. The aim was to evaluate the usefulness of the chosen imaging and image analysis strategies and to characterise the boundary conditions for successful image processing and analysis, including an articulated atlas for image registration of follow-up studies. Significant results were also obtained in deblurring and segmentation of images from dynamic series. Applying advanced techniques allowed an objective optimisation of the compromise between denoising efficiency and information conservation on dynamic contrast enhanced MR images after contrast agent injection.

The main results achieved on novel imaging reporter probes are the **synthesis of iron oxide nanoparticles at gram scale** and the **synthesis and in vitro testing of an MRI reporter based on detection of beta-galactosidase activity**. In addition, a novel cell labelling procedure was developed based on the targeting of Exofacial Protein Thiols by a specific Gd(III) complex. Production of the ligands for Gd(III) was scaled up to multigram amounts, and preparation of their complexes and methods for their analysis were reached. A new cell labelling procedure based on the use of Gd-loaded liposomes was set up.

Preliminary experiments were successfully carried out regarding novel tools for cell labelling and cell fate imaging (recruitment, differentiation, and cell death). Initial **cell labelling experiments** indicated the ability of porphyrins incorporated in endosomal membranes to induce the endosomal escape of contrast media by UV irradiation of labelled cells. Studies performed on cell labelling strategies indicate that general principles regarding cell labelling protocols may be defined. However, it appears that **optimal protocols will have to be tailored for each combination of imaging probe and cell type**. A literature review on cell labelling protocols for paramagnetic probes was performed and is now in press at the journal European Radiology. Another highlight is the successful construction of the Adenovirus TK vector. As for **cell fate imaging**, performed experiments showed that it is possible to image tumour stroma cell activation and differentiation by fluorescence intravital microscopy.

In terms of pre-clinical validation, the main results related to **neurological diseases** were the design of paramagnetic CEST agents with improved properties for cell labelling purposes. A transgenic mouse model was generated. The model reports on neurogenesis using a bioluminescent reporter allowing an increase in neurogenesis to be detected after stroke. As for **cardiovascular diseases**, studies show that ungated 3D GRE acquisitions can provide time-efficient and sensitive detection of SPIO labelled cells relative to 2D CINE scans and cardiac gated 3D scans. The major breakthrough related to **musculoskeletal diseases** is the use of CEST to assess GAG concentration in the intervertebral disc, which may lead to early diagnosis of disc degeneration and a way to monitor repair mechanisms by stem cells. As for **diabetes**, one subfamily of dual modality contrast agents based on second generation dendrimers was found to be toxic to cells. However, a cyclic analogue with high relaxation properties was found to be well tolerated. The labelling of stem cells was verified by fluorescent microscopy and in vivo tests on rat models have been started. The method of semiautomatic and automatic calculation of the number of pancreatic islets was tested and is now ready for routine application. A paper with a detailed description of the procedure was published in Magn Reson Mater Phy, DOI 10.1007/s10334-009-0172-4, published online on April 24, 2009.

The major achievement in **dendritic cell therapy** studies was the observation of DC migrating to draining lymph nodes in patients enrolled in a Phase I Clinical Trial using Indium-111 scintigraphy. Clinical efficacy evaluation is pending. Only a small proportion of injected DCs emigrated from the injection site. MR imaging allowed assessment of both accurate DC delivery and inter-/intranodal migration patterns.

Pre-clinical T-cell therapy studies resulted in the successful generation and expansion of CTL that kill OVA-peptide positive target cells at 80% (MEF-OVA) and 50% (B16-OVA) efficacy.

With regard to **monitoring the dynamics of tumour and tumour microenvironment**, apoptosis was detected using a histone H2B/GFP construct, and a quantitative nuclear condensation score to discriminate apoptosis from necrosis was developed. This approach will be valuable as third colour to monitor the tumour stroma in the projected dual-colour reporter mouse. In addition, significant impact of p53 expression in fibroblasts on tumour progression was detected, which is being summarised for publication.

The main results in the translation towards clinical applications refer to cancer and diabetes: With respect to **cancer**, the clinical trials that incorporate imaging techniques were approved. In the DERMA-ER-DC 06 trial, several patients already demonstrated broad response to numerous peptides prior to vaccination. Furthermore, tetramer-based 8-colour flow cytometry has become a routine monitoring assay, allowing extended phenotypic and functional characterisation of T-cell subpopulations. The identification of polyfunctional T-cells was of particular interest, since these have been shown to elicit more effective immune responses in HIV vaccination trials. With respect to **diabetes**, a sequence for the measurement of high resolution MR images at 3T Imager Trio Siemens was developed. The position of labelled pancreatic islets was found to be visible as black spots.

Expected final results and their potential impact and use

In the long-term, ENCITE should lead to extensive collaboration on the development of novel imaging tools and implementation at the level of translational medicine across Europe leading to a significant global impact. The ultimate goal is to **see cellular therapy and image guidance enter widespread clinical practice**, offering **potential cures for many types of cancer, infectious and other diseases for patients worldwide**.

In view of the results of the first year within novel imaging technologies, the working group is confident of reaching the overall goal to provide novel imaging technologies and postprocessing tools that will enable more efficient and sensitive diagnostic and scientific tools in the field of cell therapy. Given the broad range of applications, the difference in the species observed (from mouse to man), the range of field strengths (3-11.7T) and the different platforms, it is anticipated that the planned toolbox will consist of a flexible set of protocols that can be adapted to specific studies. The consortium is **highly encouraged to facilitate new and groundbreaking developments in the field of cell-based therapies**.

The first results obtained in the field of novel imaging reporter probes are extremely encouraging with respect to the final goal of providing highly sensitive agents and procedures for an efficient labelling of cells. The **novel cellular labelling procedures are definitively more effective than the ones currently used** and are expected to have a high impact on the further development of the project.

The methods being developed for novel tools for cell labelling could potentially improve monitoring of cell therapy. Through the validation and implementation of the developed imaging tools in pre-clinical settings, a **better understanding of the fate of transplanted cells** and of how cell-based therapies provide therapeutical benefit will be obtained, and tools for clinical monitoring of such therapies can be implemented. Through the knowledge obtained in these studies, **treatment strategies can then be (further) optimised to reap full benefit of the therapeutic potential** of cell-based therapies.

Illustration and promotion of the work

The newly-designed **project logo and website** give the ENCITE project a visible face and provide appropriate access to ENCITE on a global level. To raise awareness of ENCITE within the European community, a number of promotion and dissemination activities were launched, including the distribution of **informative material to scientists, media and the general public, publications on initial scientific results** and **presentations of first milestones** by ENCITE researchers at different international events relevant to the biomedical imaging community.



ENCITE Consortium
Kick-Off Meeting, Freising/DE, June 2008



ENCITE Educational Workshop on Molecular
Imaging
Prague/CZ, May 2009

This Large Integrated Project was submitted to the call HEALTH-2007-1.2-4 “In vivo image-guidance for cell therapy” and is funded by the European Commission within the 7th Framework Programme with a financial contribution of €12m. The 4-year project started in June 2008. The overall objectives will be achieved thanks to a strong collaboration of 19 European institutes and two from Israel with outstanding scientific expertise in cell imaging, with additional partners being integrated as of the second project year.

For more information please visit the project website or contact:

EIBIR gemeinnützige GmbH, Neutorgasse 9, 1010 Vienna, Austria, office@eibir.org, www.encite.org

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