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Various Applications of Magnetic Resonance Imaging in Rodent Phenotyping

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INTRODUCTION

Animal models which include mice and rats are frequently used in the study of inflammatory, neurodegenerative, and neoplastic and vascular diseases (Denic, 2011). Small animal models of human diseases are currently needed and have become an important tool for biomedical research. Animal studies as models for human diseases provided important understandings into the aetiology of diseases and can be used to provide improvement on the current therapeutic strategies (Lo, 2017). In order to test the efficacy of experimental therapeutics and disease pathogenesis, detailed phenotypic characterisation is really important in animal models and mechanistic studies (Denic, 2011). Improvement in diagnostic accuracy, determination of prognosis, and selection of optimum treatment allowed for the more advanced imaging methods (Glodek *et al*., 2016). Magnetic Resonance Imaging (MRI), considered one of the most versatile non-invasive imaging modalities and as a transitional stage for *in vitro* and *in vivo* studies (Glodek *et al*., 2016).

Why Animal Studies are Necessary in Biomedical Research

Research using animals has played an important part in nearly every medical discovery over the last decade. Currently, animal models in research are important for the improvement and used as effective methods for diagnosing and treating diseases in both humans and animals. The experimental animals used in research to develop the model on human, in learning more about health problems and to assure the safety of new medical findings. Researchers need to understand health problems and issues before developing new treatments and therapies for human.

Scientists also use non-animal models for research whenever it is possible. There are a number of other non-animal research methods used in today's biomedical research such as computer models, cell and tissue cultures. In the beginning of the experiment, computer models are typically used to screen, monitor and determine the toxic level of substances in tissue and cell cultures. This is become a valuable addition to the array of research tools and techniques. Nevertheless, there are some of diseases and health problems that involve procedures or methods that can only be studied in animal models or living organisms (Driehuys, 2008).

Humans and animals have similar biological functions which perform the same task and similar organ systems (https://ca-biomed.org/wp-content/uploads/2018/02/FS-Laws.pdf). The genetics similarity between a mouse and a human is about 96% thus making the mouse effective model for human body. As the animal has a shorter life cycle than humans, this enables it to be studied throughout its entire life cycle which continuously through number of generations (California Biomedical Research Association). However, the use of animal in biomedical research is under control and strictly reviewed by the Committee of Animal Research and Ethics (CARE) for ethics approval to safeguard responsible research with animals and ensure ethical conduct during the study.

Small-animal Imaging

The most common approach in biomedical research is to visualise tissue compartments or cellular interactions in various tissues. In this context, a standard optical microscopy has two major limitations: 1) only *ex vivo* tissues can be studied at high resolutions, and 2) the degree of specificity varies depending on the staining

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method used (Denic, 2011). As a consequence, new approaches have been developed, such as small animal imaging to provide new methods for drug discovery and development, diagnosis, therapy monitoring, and prognostic abilities. Small-animal imaging has been recognised as an important tool in pre-clinical research, and it is undergoing continuous refinements. Since most disease processes are dynamic, non-invasive *in vivo* imaging modalities are of great advantage as they enable investigations to be performed at multiple time points. Small-animal MRI studies are usually pre-clinical; they establish or study new aspects of a disease process that have not yet been clarified in the human disease. Small-animal MRI also allows for translational projects in which the patho-mechanism of human MRI findings are studied (Glodek *et al*., 2016).

Magnetic Resonance Imaging for Animal Research

Small-animal magnetic resonance (MR) provides a higher signal-to-noise ratio (SNR) and microscopic resolution of imaging system. This system is equipped with high-field-strength magnets ranging from 3-14 Tesla. Generally, magnetic resonance image quality mostly depends on the number of inter-dependent factors in any kind of imaging systems. The difference in field strengths provide different contrast physical appearance of live tissue. High SNR signals can be achieved by lowering spatial resolution or with longer imaging times, while resolutions up to tens of microns can be achieved in living animals. MR imaging has the ability to study living organisms without exposing them to potentially harmful ionising radiation. Slice-based and true 3D image sets are available depending on the technique used. Scans of MR images were obtained in several ways, and different pulse sequences were used as the primary determinants of tissue contrast. In addition to anatomical imaging, MR is capable of providing physiological information on several important aspects of biological processes, including cerebrospinal fluid flow and flow distribution of cerebral blood volume, activity mapping with functional MRI, distribution of metabolites by chemical transition imaging, or *in vivo* MR spectroscopy. Contrast adjustments can increase the SNR, where a recent study of cell detection uses superparamagnetic oxide particles to detect single cell particles (Acton, 2006).

Uses of Magnetic Resonance Imaging

The Rodent Imager-Magnetic Resonance Imaging system was developed to support the data for the following on-going research activities at the Malaysian Palm Oil Board (MPOB).

Bone research – arthritis, inflammation. In orthopaedics, pre-clinical MRI may be used to examine bones, joints, and soft tissues of the rodents, such as cartilage, muscles, and tendons for injuries or the presence of structural abnormalities or certain other conditions, such as tumours, inflammatory disease, congenital abnormalities, osteonecrosis, bone marrow disease, and herniation or degeneration of discs of the spinal cord. MRI may be used to assess the results of corrective orthopaedic procedures. Joint deterioration resulting from arthritis can be monitored by using magnetic resonance imaging (Tremoleda *et al*., 2011).

Wound healing. The measurement of wound is important in determining the progress of wound healing in all clinical practices. The objective data such as the measurement of wound healing needed in establishing the progress of wound. MRI technique can be applied to monitor wound healing process as it gives superior imaging capability in soft tissues. Assessment on wound angiogenesis process could be one of the research applications to which the MRI technique can contribute.

Cancer research. Most of the cancer treatments in the world are under research or investigation and most of it still finding for cure. It took many years to establish the treatments, and a wide range of research techniques have been used in order to understand and to study on how the physiology of body works and diseases progress. There is a great scientific consensus around the world that some research on the use of animals is still important for medical advancement. Biologically, animal models in research have become an important tool for studying human diseases such as cancer. Determining the status of a disease is difficult due to the anatomical location of various organs such as brain, pancreas, and prostate. Imaging modalities such as MRI can improve diagnosis, and tumour longitudinal imaging is an important source of experimental data. Imaging can be performed even in remote areas and allows for the determination of visual tumorigenesis, anatomical imaging and longitudinal function to improve the scope of the study by facilitating the assessment of biological changes (such as changes in angiogenesis, metabolism, and cellular invasion) as well as tissue perfusion and dissemination (James, 2013).

Functional MRI is a rapidly developing tool for non-invasive assessment used in oncology. This is particularly for the improvement or advancement in therapy

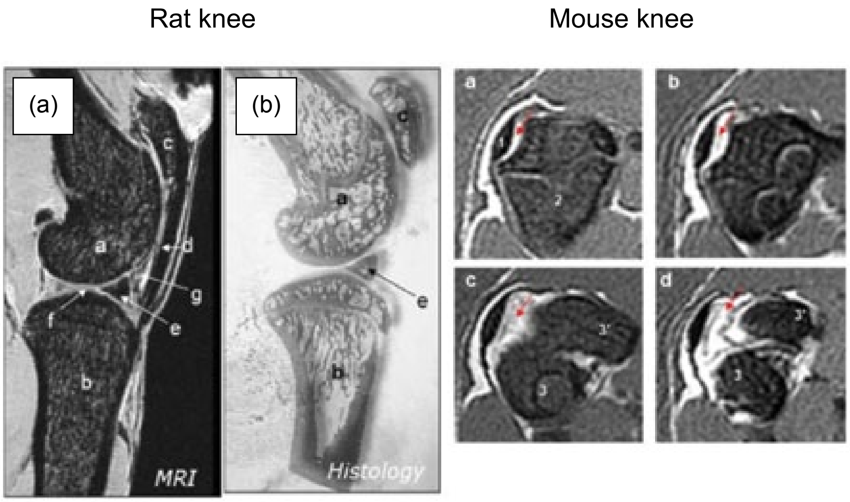

Figure 1. The soft tissue which surrounds the rodent's knee joint can be imaged by using the MRI technique as compared to the histological model. The above are MRI images of a rat (Wistar) and mouse (C57BL/6) knee joint (Tremoleda et al.*, 2011).*

especially for diagnostic and monitoring purposes (http://www.mrsolutions.com). Up-to date, clinical techniques are currently on microcirculation imaging using gadopentetate dimeglumine and analogues, the extracellular low molecular weight contrast agents (http://www.mrsolutions.com). Time-based evolution is used to improve the lesions of angiogenic properties such as vascular density, permeability heterogeneity, and changes during therapy (Knopp *et al*., 2003).

The following are the ideal techniques for:

- Finding a tumour
- Determining tumour nature
- Evaluating the cancer stage (size and position of the tumour)
- The planning for treatments, surgery and radiation therapy
- Monitoring of the tumour responses after treatment
- Evaluating lymph node
- Tumour angiogenesis
- Estimating the possibility of relapse/recurrent disease

Antioxidant research. A few studies have reported the use of pre-clinical MRI to determine the effect of antioxidants on knee cartilage and bone in rodents (Md Nazrul and Pervin, 2011, Fiala *et al*., 2015). Antioxidants are molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, which lead to a chain reaction that can deteriorate cells. Antioxidants such as thiols or ascorbic acid (vitamin C) and tocotrienols can be used to eliminate this chain reaction (Md Nazrul and Pervin, 2011, Rink and Khanna, 2011).

Recent study from The Federation of American Societies for Experimental Biology (FASEB) Journal suggests that clinical trials of omega-3 and antioxidant supplementation should be undertaken by people who have Alzheimer disease with mild clinical impairment. Besides, there is also a research study which determined whether or not antioxidants (vitamin E, selenium, and lycopene) can change (reduce) prostate tumour size or blood flow to the prostate as shown in rodent MRI imaging (Fiala *et al*., 2015).

Toxicology studies. Drug development studies almost always begin in the test tube-or more probably, the microtiter plate. However, if a compound is ever to transition to the clinic, it must move from the microplate well to animals. Such pre-clinical studies are a must in pharmaceutical development, as compounds that look promising in a cell culture dish may not work in animals or may produce unexpected and undesirable side effects (Perke, 2012).

Cognitive function. MRI is increasingly being used in the studies on anatomical or morphological changes (especially in gray matter atrophy) in Parkinson's disease and other neurodegenerative disorders as well as non-alcoholic fatty liver disease (NAFLD). Moreover, MRI is used to map the motor and cognitive substrates of neurobiology in Parkinson's disease through functional (blood-oxygen-dependent) MRI. The study conducted using MR spectroscopy (MRS) imaging has shown a decrease in N-acetyl-aspartate-to-creatine ratio in striatum specimens with Parkinsonism compared with controls (Waerzeggers *et al*., 2010).

Immunology responses. Non-invasive cell tracking is the latest approach for imaging cells in their native environment. The characteristic of the immune cells is defined by the shape and level of expression of cell surface molecules and their secreted factors. These molecules are usually tested *in vitro* using techniques such as flow cytometry and immunohistochemistry (Ahrens and Bulte, 2014).

Other than that, through a non-invasive imaging system such as MRI, the important characteristic and phenotype of cell populations in the immune system can be identified through insertion of labeling of dyes and image monitoring without sacrificing the animals (Ahrens and Bulte, 2014).

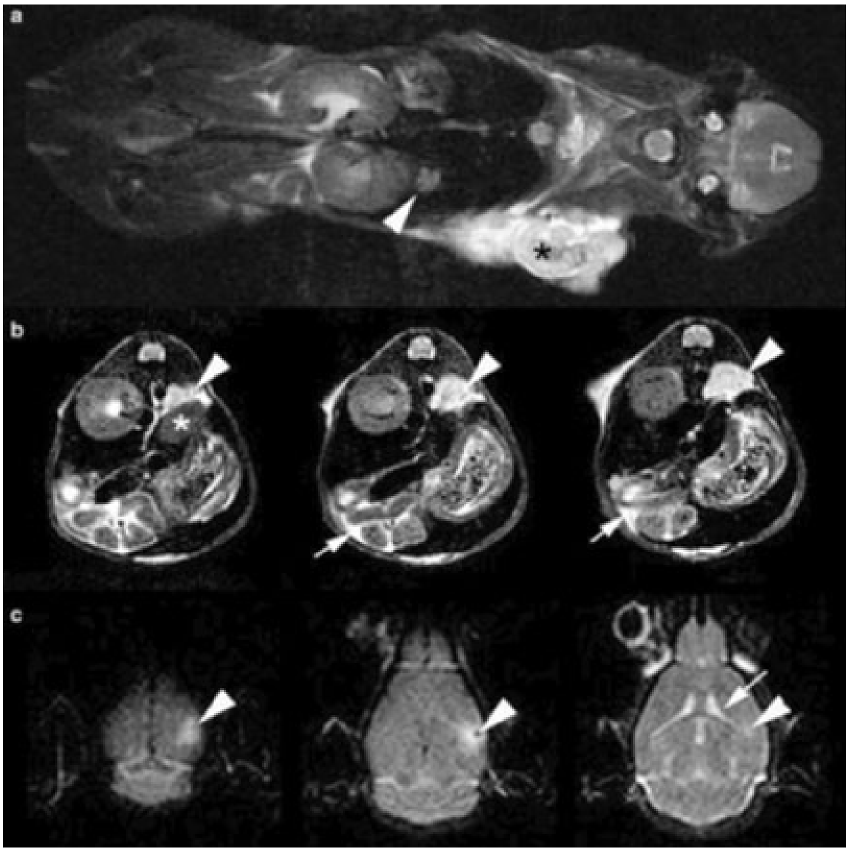

Figure 2. Magnetic resonance imaging of a xenograft mouse model of the human melanoma cell line FEMX-I examined 11 weeks after surgical excision of the primary tumour (Peldschus and Ittrich, 2013).

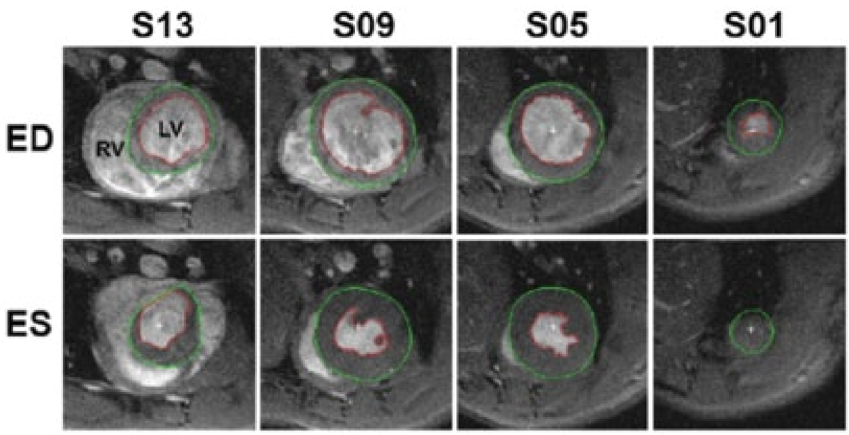

Figure 3. Segmentation of a typical cine stack of a rat heart. The red line delineates the endocardial border, whilst the green line marks the epicardial border.

The increasing complexity in *in vivo* imaging technology together with the increase of cell therapy has led to a revolution of immune cell detection *in vivo*. Development of MRI methods using iron oxide probes and 19F is currently being developed. This MRI technology can be used for the transmission of image-guided immune cells and visualisation of immune cells and engraftment, inflammation, cell physiology, and gene expression. In addition, MRI-based cell tracking is currently being used to evaluate therapeutics that modulate endogenous immune cell recruitment and monitor newly emerging cellular immunotherapy. Recent studies show that MRI has the potential to be developed in many applications to monitor the immune cells *in vivo* (Ahrens and Bulte, 2014).

Cardiovascular. Cardiac MRI is one of the important technologies in medicine for non-invasive assessment of the cardiovascular system. The system is based on the same basic principles as MRI, but with optimal use in cardiovascular system. Optimisation technique in this system mainly used for electrocardiographic (ECG) gating and rapid imaging sequences. The cardiovascular system can be evaluated or measured through various techniques by combining the key functional and morphological features. Cardiac MRI produces both still and moving pictures of the heart and major blood vessels. Clinicians use cardiac MRI to capture the beating heart and analysing the structure and condition. All these pictures help scientists choose or select the best treatment for people who suffer from heart problems (Lee *et al*., 2018).

Current Application of Small Magnetic Resonance Imaging at the Malaysian Palm Oil Board Pre-clinical Facility

The MPOB facility is equipped with a 3.0 Tesla MRI from MR Solution. The scanner is equipped with micro-imaging of animal models called as Rodent Imager. The MRI system provides superior soft tissue dissimilarity, gap and molecular imaging competency with cryogen-free superconducting and dry magnet enabling variable field strengths experiment from 0.1T to 3T and is safe to be used at any facilities. *Figure 4* shows the pictures of MPOB MRI scanning facility and the room that is equipped with an electronic rack and a working bench with a computer for image analysis.

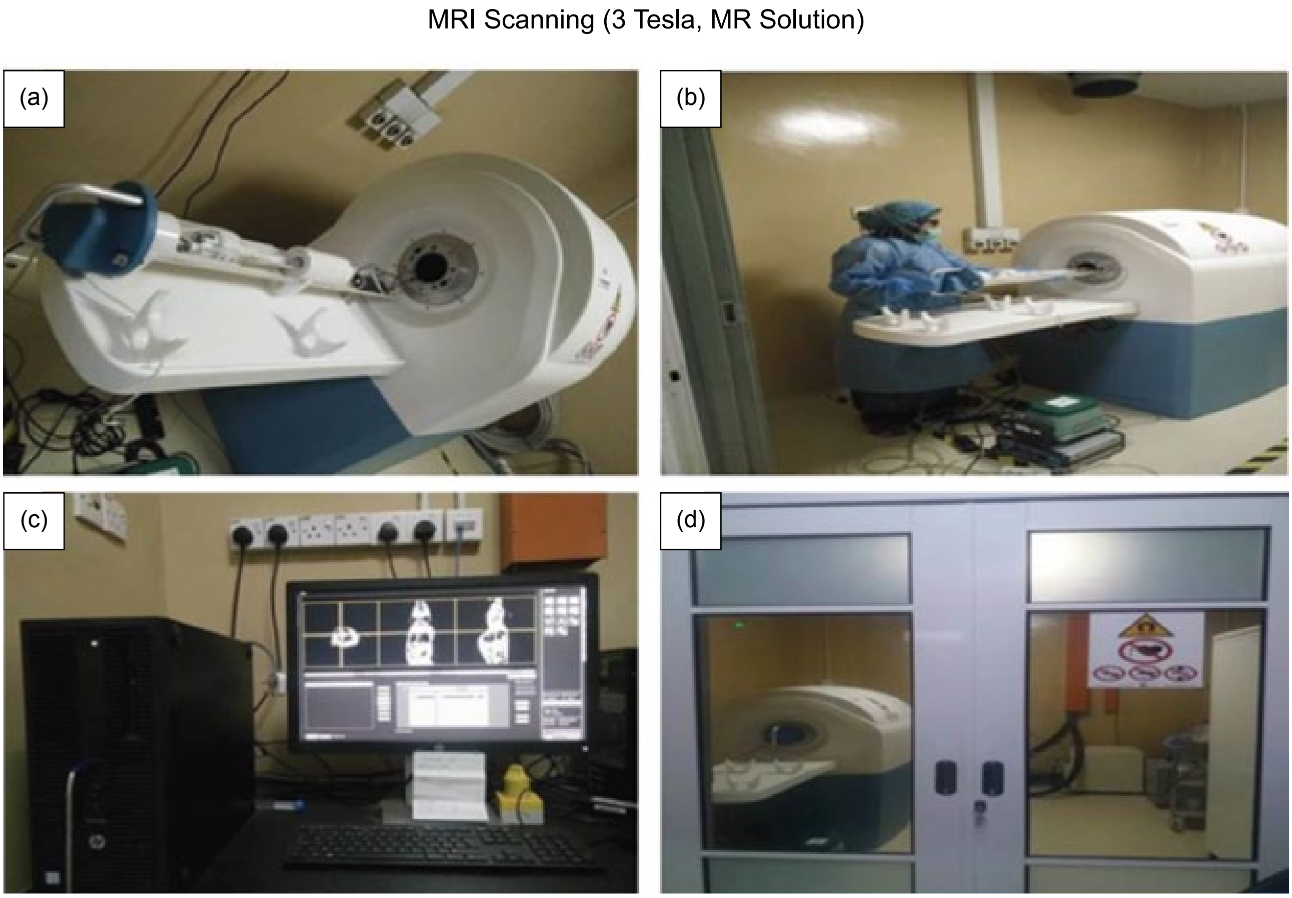

Figure 4. The pictures show the MRI facility at The Pre-clinical Building. a) The MRI benchtop site for animal scanning. b) A staff placed an anaesthetised animal into the benchtop MRI site which was ready for scanning. c) The working bench for analysing the image of animal during scanning. d) The room for MRI scanning which is located at the Specific Pathogen-Free Unit.

Sections in animals	Divides the organism in
Coronal/frontal	A dorsal and a vetral part one each side
Sagittal/length	Left and right side (Median section is exactly in the middle)
Transversal/axial	A caudal and a cranial part

In order to demonstrate the effectiveness of the images from the MRI imaging scanner in our facility, we used a group of mice on which were the new 4T1 cell lines (mammary cancer cells) that had have been purchased recently were tested. The image sections were prepared for analysis as shown in *Figure 5*. The BALB/C mice were divided into three groups, namely the negative control, the positive control, and the treatment. The mouse in the negative control group did not receive any tumour injection, whereas the mouse in the positive control received 10^3 of 4T1 cells through subcutaneous (SC) injection into the mammary fat pad without any treatment. For the treatment group, the mouse received 103 of 4T1 cells and was supplemented with 1 mg/ body weight (kg) of TRF through oral gavage (*Figure 6*). In week 4, all the mice were scanned for tumour growth and to compare the images between groups. All the mice were anaesthetised with isoflurane before the scans were conducted. The mice heart rate was monitored throughout the scanning. The amount of isoflurane given was just enough for a 30 min analysis conducted using MRI. Each scanning needed 1-3 min before the full image could be gathered.

The MRI results in *Figure 6* show the differences between control and treated mice. The MRI image scan showed no tumour in the negative control in the axial and coronal planes of mouse body image, whilst there is a large tumour was found in the positive control group. We observed that the MRI image showed smaller tumours developed in the treated mouse compared to the positive control. In addition, size of tumours could also be measured using the virtual calliper provided from the analytical software.

CONCLUSION

The MRI provides an excellent platform for translational medicine

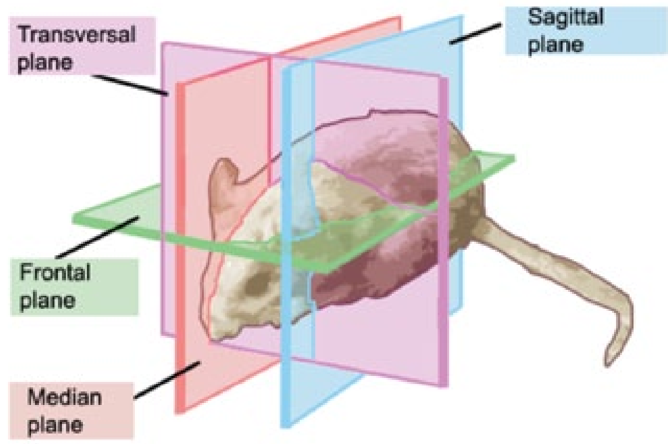

Figure 5. Anatomy orientation of 3D image for mouse using in MRI. The figure is adapted from Terminology 3D-orientation. Website: https://www.vcbio.science.ru.nl/en/3d-orientation.

tumour detection, brain imaging, organ imaging, monitoring gene expression, functional analyses, protein interaction analysis and determining pharmacokinetics as well as for investigating new contrast mechanisms and agents. It can be used to track the progression of a disease or intervention in the same living animal, thus improving a study's biological and translational applicability.

The MRI system at MPOB enables multi-modality imaging and can be upgraded with nuclear medicine imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). The combination of these

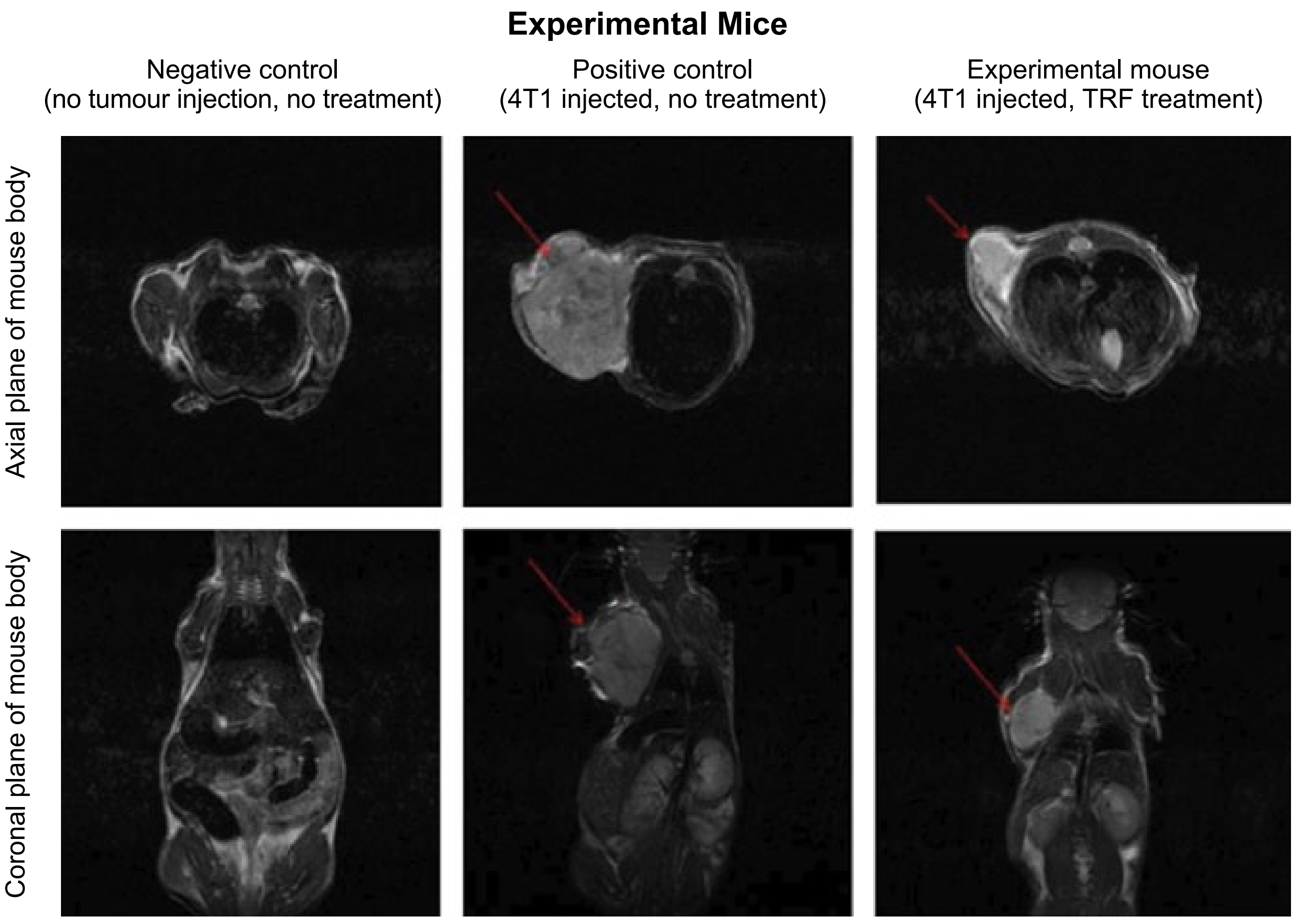

Figure 6. Experimental mice used to study the effect of TRF on tumour formation and growth. a) Negative control (no tumour, and no treatment) introduced to this group. b) 4T1 injected into the mammary fat pad of mouse. No treatment given. c) Experimental mouse, 4T1 injected into the mammary fat pad and 1 mg of TRF was given every day through oral gavage. The red arrows point to the tumours that developed in the mice. The mice were analysed using axial and coronal planes of the body images.

and pre-clinical research. The greatest advantage of MRI in small-animal research studies are non-invasiveness technique which allow for anatomical, pathological, and functional information to be obtained repeatedly over time and in shorter period. It can be used in various research applications, such as assessments of disease progression, techniques and scanner will provide detail on the anatomical and metabolic information. The system is also compact with a small footprint design which can be placed in close proximity to other imaging modality in the future.

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REFERENCES

Acton, P D (2006). Animal imaging equipment: Recent. *The J. Nuclear Medicine*, *Vol. 47, No. 12.*

Ahrens, E T and Bulte, J W M (2014). Tracking immune cells in vivo using magnetic resonance imaging. *Nat. Rev. Immunology. 13(10)*: 755-763.

Denic, A S I (2011). MRI in rodent models of brain disorders. *Neurotherapeutics*. *8(1)*: 3-18.

Driehuys, B J N (2008). Small Animal Imaging with magnetic resonance microscopy. *ILAR Journal*, *49(1)*: 35-53. DOI: 10.1093/ilar.49.1.35.

Fiala, M R; Halder, R C; Sagong, B; Ross, O; Sayre J; Porter, V and Bredesen, D E (2015). Supplementation increases amyloid-phagocytosis and resolving D1 in patients with minor cognitive impairment. *The FASEB Journal*. *29(7)*: 2681-2689. DOI: 10.1096/fj.14-264218.

Glodek, J; Adamiah, Z and Przeworski, A (2016). Magnetic resonance imaging of reptiles, rodents and logomorphs for clinical diagnosis and animal research. *Comparative Medicine*. p. 216-219.

https://ca-biomed.org/wp-content/uploads/2018/02/FS-Laws.pdf. California Biomedical Research association page

https://www.vcbio.science.ru.nl/en/3d-orientation. Anatomy orientation of 3D image for mouse using in MRI.

James R M (2013). Multispectral imaging: A review of its technical aspects and applications in anatomic pathology. *Veterinary Pathology, 51(1)*: 185-210.

Knopp, V; M V; Von Tengg-Kobligk, H and Choyke, P L (2003). Functional magnetic resonance imaging in oncology for diagnosis and therapy monitoring. *Molecular Cancer Therapeutics, 2(4)*: 419-426.

Lee, D C; Markl, M; Dall'Armellina, E H; Kozerke, Y; Kuehne, S; Nielles-Vallespin, T; Messroghli, S; Patel, D and Amit (2018). The growth and evolution of cardiovascular magnetic resonance: A 20-year history of the Society for Cardiovascular Magnetic Resonance (SCMR) annual scientific sessions. *J. Cardiovascular Magnetic Resonance 20(1)*: 8. DOI:10.1186/s12968-018-0429-z. ISSN 1532-429X. PMC 5791345. PMID 29386064.

Lo, Y L (2017). Diverse application of magnetic resonance imaging for mouse phenotyping. *Birth Defects Res*. *109(10)*: 758-770.

Md Nazrul, I and Pervin, S (2011). Review article anti-oxidants. *Dhaka National Med. Coll. Hos. 17 (02)*: 61-64.

Peldschus, K and Ittrich, H (2013). Magnetic resonance imaging of metastases in xenograft mouse models of cancer. Part of the Methods in Molecular Biology Book Series. *Metastasis Research Protocols*. 1070: 213-222.

Perke, J M (2012). *In vivo* toxicology imaging: Moving compounds from the microtiter plate to the clinic. *Life Science Article.* https://www.biocompare.com/Editorial-Articles/119741-Preclinical-Imaging/ Research using animals: An overview (http://www.ox.ac.uk/news-and-events/animal-research/research-using-animals-an-overview).

Rink, C and Khanna, S (2011). Significance of brain tissue oxygenation and the arachidonic acid cascade in stroke. *Antioxid Redox Signal*, *14(10)*: 1889-903. DOI: 10.1089/ars.2010.3474.

Tremoleda, J L; Magdy Khalil; Luke L Gompels; Marzena Wylezinska-Arridge; Tonia Vincent and Willy Gsell (2011). Imaging technologies for preclinical models of bone and joint disorders. EJNMMI Res 1: 11. DOI:https://doi.org/10.1186/2191-219X-1-11

Waerzeggers, Y; Monfared, P; Viel, T; Winkeler, A and Jacobs, A H (2010). Mouse models in neurological disorders: Applications of non-invasive imaging. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 1802(10)*: 819-839. DOI:https://doi.org/10.1016/j.bbadis.2010.04.009.