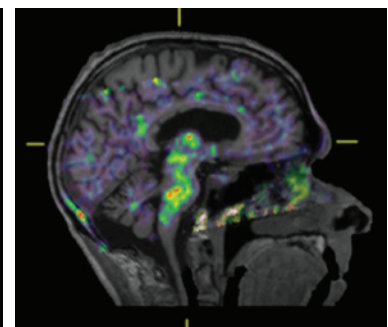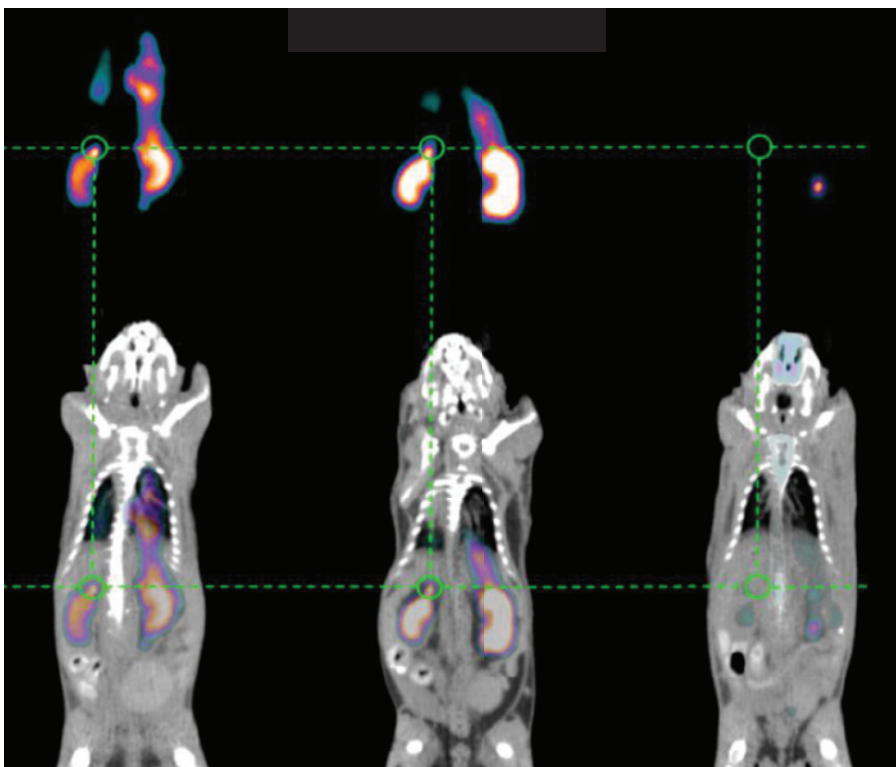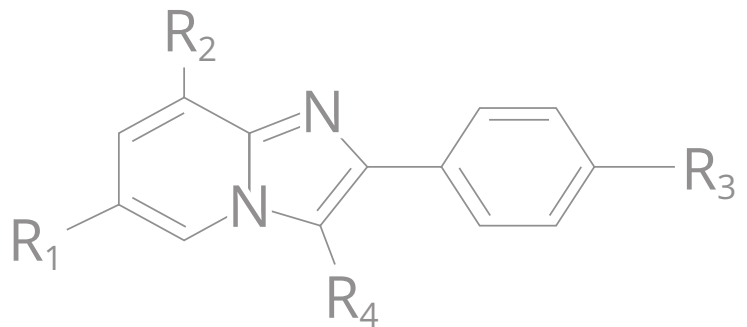# TSPO (18 kDa) knockout

A real life tool for rational drug design

In vivo – testing of:

- Compound specificity and selectivity
- Mechanism of action

+/+   +/-   -/-

TSPO
Translocator Protein (18 kDa)

Highly conserved mitochondrial outer membrane protein associated with steroid hormone production, transport of cholesterol into the mitochondria, energy metabolism and the regulation of inflammatory immune responses.

The Translocator Protein (TSPO)

The mitochondrial translocator protein (TSPO) knockout (C57BL/6-*Tspo*<tm1GuWu>) is a mouse model developed at the **Australian Nuclear Science and Technology Organisation (ANSTO)**, Australia's leading nuclear science and research organisation, in collaboration with the **Brain & Mind Research Institute, The University of Sydney**.

Its purpose is to enable the conclusive testing of selectivity and mode of action of TSPO-binding compounds with diagnostic and therapeutic potential. This approach can be applied to the development of new chemical entities as well as the repurposing of existing drugs.

For the first time, observations made in reductionist models, such as cell culture, can be translated to a widely used animal model of human physiology and verified unambiguously in a complex mammalian system.

> "The paper of R.B. Banati and co-workers is definitely a breakthrough… (it) rejuvenates basic science around TSPO and the potential design and development of drugs interacting with this target."
>
> **Gerard Le Fur**
> Académie des Sciences, France

The paradigm shift

Pathways of the innate immune system are involved across many acute and chronic diseases

Understanding inflammatory or immune-mediated pathways allows mechanism-based diagnosis and therapy across organ/systems and disease classifications

Broader indications for drugs targeting these pathways increases clinical return on investment

This TSPO knockout mouse model closes an important gap in the tool kit available to study conditions in which the TSPO or TSPO-binding compounds are physiologically relevant and promise therapeutic potential, including:

- Behavioural and psychiatric disorders, such as anxiety disorders;
- Obesity, diabetes and metabolism disorders;
- Neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease;
- Inflammatory brain disease, such as multiple sclerosis (MS);
- Inflammation-associated pain;
- Cancer biology.

The future of research and development into TSPO

TSPO, previously known as the peripheral benzodiazepine receptor (PBR), is a mitochondrial outer membrane protein that is highly conserved across species from bacteria to mammals. The TSPO is associated with crucial cellular functions: steroid hormone production, transport of cholesterol into the mitochondria, as well as energy metabolism and the regulation of inflammatory immune responses.

TSPO expression levels have been shown to be highly increased in a variety of disease states and to correlate with the severity of illness and rate of illness progression. Over the last fifteen years, research into the TSPO as a widely applicable diagnostic and therapeutic target has intensified significantly. As of 2014, there have been well over 30 clinical trials involving the measurements or the therapeutic targeting of TSPO in disease conditions ranging from inflammation to neurodegeneration and behavioural illnesses.

The use of the TSPO knockout mouse model will allow for the molecular function of TSPO in disease to be understood in greater detail, and be tested *in vivo* under realistic and controlled conditions. This is a crucial step towards the rational and accelerated development of next generation therapeutics targeting TSPO related functions in disease.

Partnership opportunities

ANSTO recognises that effective partnering can accelerate realising the potential of discoveries, turning them into innovative products more quickly and at reduced cost than would occur via purely in-house development. ANSTO is therefore, actively seeking collaborative partners to use the TSPO knockout mouse model (C57BL/6-$Tspo^{tm1GuWu}$) to determine the action of new compounds, including the repurposing of existing compounds and development of novel therapeutic approaches.

Business partnerships

Due to the rapid development of the TSPO field and its anticipated impact on drug development, ANSTO is actively seeking to engage with partners in the biotechnology sector and pharmaceutical industry. ANSTO welcomes licensing arrangements and other business collaborations with industry to ensure the TSPO knockout mouse model is utilised and actively contributes towards the development of TSPO compounds with validated specificity and selectivity.

As the TSPO has significant implications in a variety of disease states, ANSTO is looking for commercial relationships with companies and/or individuals whose strategic priority is in the areas of anti-inflammatory and anti-analgesic therapeutics, and areas of development such as anxiety, diabetes, obesity and cancer.
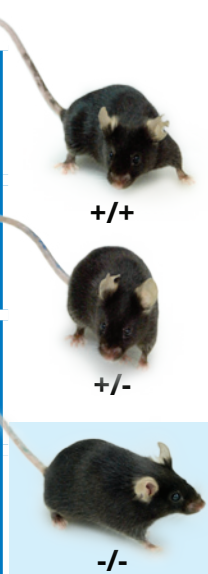
Research partnerships

ANSTO is now accepting expressions of interest from researchers and industry in relation to research and technology developments where the TSPO knockout mouse model can provide valuable insights. ANSTO LifeSciences has a keen interest in exploring diagnostic and therapeutic indications relevant to the TSPO and in forming collaborative partnerships to initiate programs of compound development or repurposing of existing compounds.
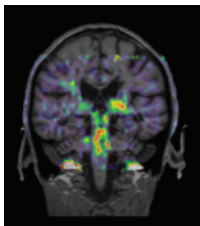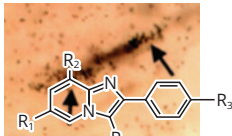
Partnership opportunities

TSPO KO mouse	Ligand screening	Drug development	Preclinical	Clinical trials
Strong IP portfolio to develop collaborations	TSPO KO and wild-type mice to evaluate TSPO ligands (ligand does or does not bind to TSPO)	Validate the biological pathways involving the TSPO. Identify lead compounds which have therapeutic effects	Lead candidates developed using the TSPO KO mouse are established or are being developed in a preclinical environment	Trials of drug candidates for potential registration

Areas of scientific inquiry

Inflammation	Behavioural and psychiatric disorders
Diabetes and obesity	Cancer

+/+

+/-

-/-

Applications

Diagnostics

Therapeutics

Repurposing

New development

Applications

The TSPO knockout mouse model (C57BL/6-*Tspo^{tm1GuWu}*) developed at ANSTO is useful in the study of various disease states. It is the most advanced and characterised *in vivo* method to acquire robust specificity and selectivity data for TSPO-binding compounds. It enables faster evidence-based decisions in the selection of suitable drug candidates for the TSPO and unique insights into the mechanism of action or off-target effects.

- Accelerated drug development
- Further understanding of the mechanisms of action
- Evidential proof to strengthen end product value

Inflammatory disease

Pathways of the innate immune system have broad relevance for the diagnostics and therapeutics of many acute and chronic diseases.

The TSPO knockout mouse model, as shown in Banati et al., (*Nature Communications* 2014, in press) allows the dissection of the TSPO associated inflammation pathways.

There is increasing recognition that glial responses are underpinning regeneration and more careful separation of inflammatory pathways from neuro-glial interactions is needed to design rational therapeutic strategies.

The TSPO has been under investigation as a drug target for inflammatory conditions, such as arthritis and MS. In the case of MS, activated microglia are involved in the immune response. The TSPO is expressed by microglia and up-regulated in MS, particularly in the case of secondary progressive MS correlating with clinical disability. TSPO ligands have been used experimentally to mitigate the course of auto-immune inflammation.

Obesity, diabetes and metabolism disorders

The use of TSPO null background material, such as the TSPO knockout mouse model, can provide the direct validation of the selectivity and specificity of many TSPO targeting compounds which have so far, been difficult to obtain.

It has recently been reported that TSPO plays a role in modulating energy, obesity and diabetes. After testing the effect of 2,400 bioactive compounds on glucogenesis using a high throughput screen in transgenic reporter zebrafish, it was discovered that TSPO ligands PK11195 and Ro5-4864 decrease glucose levels and reduce energy consumption, suggesting a role for TSPO in glucose metabolism (Gut et al., *Nature Chemical Biology* 2013, 9: 97–104). Further, it was demonstrated that PK11195 and Ro5-4864 reduced blood glucose in 24 hour fasted mice, reduced weight gain and improved glucose tolerance in a mouse model of high-fat induced obesity and diabetes. The effect of PK11195 on glucose homeostasis suggests that TSPO may have a role in cellular energy consumption through modulation of mitochondrial energy production. The utility of this approach has been already demonstrated for a number of ANSTO synthesised-TSPO compounds.

Neurodegenerative disorders

The TSPO knockout mouse model can help in determining the specificity and selectivity of a TSPO ligand, and provide answers in regards to mechanism of action and therapeutic efficacy.

TSPO expression has been used as a diagnostic biomarker of neuro-inflammation, while synthetic ligands for the TSPO are being studied as potential therapeutic drugs that alter the functional state of the brain innate immune effector cell, microglia.

TSPO ligands with a potential to ameliorate or reverse neurodegenerative pathology, such as Alzheimer's disease, are already under active investigation.

Anxiety and other psychiatric disorders

The TSPO knockout mouse model is currently the most advanced approach to determine the specificity and selectivity of anxiolytic TSPO ligands *in vivo*.

Despite new insights into underlying brain mechanisms associated with psychiatric disorders, there has been relatively little progress in the identification of novel pharmacological treatments of anxiety.

TSPO targeting ligands are one of the few novel targets for anxiolysis. A number of studies have demonstrated therapeutic promise in anxiety treatment (Rupprecht et al., *Nature Reviews Drug Discovery* 2010, 12: 971-88), in particular by minimising the risk of tolerance, dependence and sedation which are common with current anxiolytics.

Recent research discoveries implicate the TSPO in antipsychotic response and antipsychotics induced weight gain as well as suggesting that chronic fatigue syndromes are associated with TSPO up-regulation in the brain.

ANSTO, nuclear technologies and the TSPO

ANSTO is a world leader in medical isotope research and application with a particularly strong history of research and development of synthetic ligands for the peripheral benzodiazepine receptor/TSPO used in preclinical and clinical research into neurodegeneration, malignant tumours and inflammatory gliosis.

Professor Richard Banati's team has made significant contributions to the field, attracting more than a quarter of all citations, including the highest cited original scientific publication, which reported the *in vivo* expression of TSPO in neurodegenerative disease.

ANSTO and its partners are at the forefront of the fast growing field of TSPO research and application, and are leaders in the research and development of synthetic TSPO ligands, such as SPECT marker [123I]CLINDE and PET imaging agent [18F] PBR111, for diseases such as Alzheimer's, Parkinson's disease and MS.

The work on the knockout mouse model and the extensive medicinal chemistry research at ANSTO are part of a broader portfolio that ranges from structural biology using synchrotron X-ray and neutron scattering and cancer therapeutics, to environmental health studies using the largest particle accelerator infrastructure in the southern hemisphere. The integration across several advanced biomedical technology platforms within a single organisation allows ANSTO to offer unique partnerships across disciplines and industry sectors.
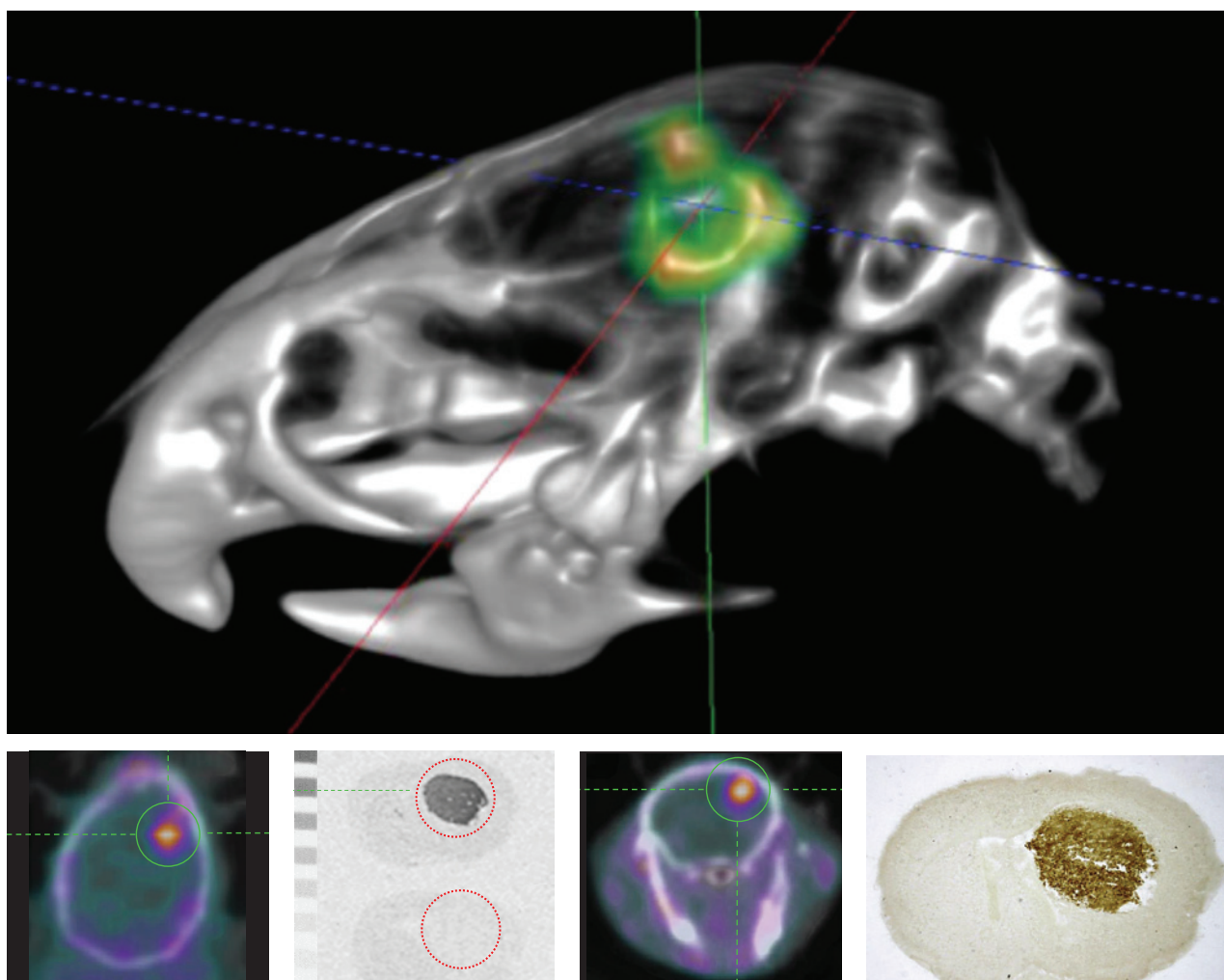
[18F]PBR111 PET/CT demonstrates the unparalleled signal-to-noise ratio achievable using the 'null background' TSPO knockout model: Selective binding is strictly confined to the implanted *Tspo*$^{+/+}$ brain tumour while the surrounding *Tspo*$^{-/-}$ brain shows no ligand binding. The binding is specific and selective and can be fully displaced.

The team

Professor Richard Banati

ANSTO LifeSciences, Sydney, Australia
BMRI, The University of Sydney, Australia

Professor Richard Banati is an internationally-recognised scientist with interdisciplinary research interests in the brain's innate immune system and the development of advanced medical imaging for the detection of subtle or non-obvious brain pathology.

He trained at the Max Planck Institute for Psychiatry, Munich, continuing at the Royal Postgraduate Medical School, London, undertaking research at the Medical Research Council MRC Cyclotron Unit at Hammersmith Hospital, Imperial College, London. Prior to becoming Professor and Foundation Chair of Medical Radiation Sciences at The University of Sydney, he held a Japan Society for the Promotion of Science (JSPS) supported professorship at the University of Kyoto.

In 2008, Professor Banati joined ANSTO as a Distinguished Research Fellow developing new bioanalytical applications of nuclear technologies.

Professor Banati is Director of the Ramaciotti Centre for Brain Imaging at the Brain & Mind Research Institute (BMRI), The University of Sydney and Director, joint The University of Sydney/ANSTO node of the National Imaging Facility.

Dr Guo Jun Liu

ANSTO LifeSciences, Sydney, Australia
BMRI, The University of Sydney, Australia

Dr Guo Jun Liu is the Biology Group Leader at ANSTO LifeSciences. His main research area is neurosciences, focusing on the role of TSPO in the central and peripheral systems. He has particular expertise in immunebiology, cell biology, electrophysiology and animal behaviour. Dr Liu began his scientific research career after he received MD and master in neurophysiology from the Norman Bethune University of Medical Sciences (now named Jilin University) in China, where he worked as Assistant Professor. After receiving a PhD in Neurosciences from the Gifu University School of Medicine in Japan, he was awarded a University Postdoctoral Fellowship by the University of Western Australia and subsequently a University Fellowship from the University of Technology Sydney. As a senior research fellow, Dr Liu continued his research in neurosciences in the Department of Physiology and the Brain & Mind Research Institute at The University of Sydney.

Dr Ryan Middleton

ANSTO LifeSciences, Sydney, Australia

Dr Ryan Middleton is a molecular biologist in the Biology Group at ANSTO LifeSciences.

Dr Middleton obtained a Bachelor of Science in Agriculture, with a focus on Agricultural Genetics, from The University of Sydney. He then went on to obtain a PhD from The University of Sydney and Johnson & Johnson Research with research focusing on the mRNA targets of MicroRNAs that are up-regulated in human disease.

Dr Middleton has extensive expertise in molecular cloning, gene expression analysis, and genetics in the context of academic research as well as the focused commercial product development.

For more information

Prof. Richard Banati
NST, Strategic Research

P +61 2 9717 3505
E richard.banati@ansto.gov.au

Lara Storr
Business Development &
New Product Management

P +61 2 9717 3855
E lara.storr@ansto.gov.au

www.ansto.gov.au