Partnered Services

7

7 Partnered Services

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7.1. Central Nervous System

The CNS services are offered in co-operation with VivaCell.

7.1.1. CNS: *in vitro* Services

Assays for the investigation of inflammation, pain, fever, depression and neurodegenerative disorders like Alzheimer's disease, multiple sclerosis, etc. are offered.

Models

- primary microglia / astrocytes / neurons (rat, murine)
- U373 MG (human astrocytoma cell line), human neuronal cell lines
- brain slice cultures
- 293T and CHO cells with heterologue expression of the Vanilloid Receptor type 1 (VR-1)

Parameters

- Prostaglandins (e.g. PGE2), cyclooxygenase expression and activity (COX-1, COX-2)
- iNOS/ NO release
- cytokines (TNF-α, IL-6, etc)
- growth factors (NGF, etc.)
- neuropeptides (substance P, etc.)
- transcription factors (NF-κB/IκB, AP-1, STATs, etc.)
- kinases (Erks, Protein kinase C, Jun, p38 MAP), etc.
- neurotransmitters (e.g. serotonin, noradrenalin, dopamin re-uptake)
- proteasome activity
- calcium mobilization, cytotoxicity and apoptosis, determination of ROS and mitochondria transmembrane potential
- agonist and antagonist of the VR1 for non-opiaceus analgesic pharmacophores
- receptor assays (screening, membrane preparations, cells, dose-response)

7.1.2. CNS: Neurophysiology

Models

- primary slice cultures of guinea pig

Parameters

- measurements of transmembraneous calcium and potassium currents in C1 pyramidal cells, interneurons and neurocortical pyramidal cells
- associative and non associative LTP and LTD in the hippocampus
- firing pattern in hippocampal and neurocortical cells
- pharmacological screening via associative LTP
- models of epileptic activity

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7.1.3. CNS: *in vivo* Services

Highly specialised and reliable preclinical *in vivo* disease models for drug development are offered.

Stroke	■ transient or permanent occlusion of the middle cerebral artery in rat, mouse, and transgenic rodents, focal ischemia in gerbils
Cardiac Arrest	■ occlusion of both carotid and vertebral arteries in rat and gerbil
Head Trauma	■ controlled cortical impact injury in rat and mouse
Spinal Cord Trauma	■ with a spinal cord impactor in rat
Parkinson's Disease	■ intracerebral injection of 6-OH dopamine in rat and mouse
Epilepsy	■ systemic / hippocampal kainic acid injection in rat and mouse
Huntington's Disease	■ intrastriatal injection of quinolinate in rat and mouse
Migraine	■ spreading depression induced by KCl in rat and mouse
Brain Edema	■ cold-injury-induced in rat and mouse

Neuromuscular and Neurological Models

- multiple sclerosis (EAE), transgenic models for ALS
- inherited murine models of motoneuron diseases

Peripher Neuropathy	■ sciatic nerve crush ■ diabetic rats (streptozotocin induction) ■ acrylamide and cisplatin-induced neuropathies
Pain	■ chronic or acute pain
Anxiety	■ free exploratory test ■ white / dark box ■ elevated plus maze ■ open-field
Depression	■ chronic mild stress ■ forced swim test
Schizophrenia	■ prepulse inhibition memory & amnesia ■ conditioned avoidance responses (passive avoidance) ■ maze learning tasks (radial maze, T-maze) ■ object recognition task ■ spontaneous and delayed alternation tasks (T-maze) ■ operant bar-press task
Anhedonia, Hedonia	■ place preference conditioning

7.2. Immunology / Inflammation

The immunology / inflammation services are offered in co-operation with VivaCell.

A wide porfolio of protocols to evaluate the natural and specific immune response in isolated human and murine lymphoid cells and also in transformed cell lines is offered. In addition, animal models mimicking human pathologies are also available. These *in vivo* and *in vitro* models are suitable for preclinical validation of anti-inflammatory and immunomodulatory compounds.

7.2.1. Immunology / Inflammation: *in vitro* Services

Models	■ primary human monocytes (rheumatic diseases, wound healing, etc.) ■ primary T-/B-lymphocytes, neutrophils, Natural Killer cells ■ primary fibroblasts, keratocytes, melanocytes ■ primary endothelial cells (heart) and HUVEC ■ murine thymocytes and primed T cells from spleen and lymphatic nodes of antigen-stimulated mice ■ enrichement of dendritic cells ■ chondrocytes ■ transformed T cell lines, B cell lines and macrophage cell lines
Parameters	■ Prostaglandins (PGE2), cyclooxygenases (COX-1, COX-2), 5-lipoxygenase and determination of leukotriens by ELISA, activity of cPLA2 ■ cytokines by ELISA or RT-PCR (monocytes: TNF-α, IL-6, IL-1, IL-8, etc.; T-cells: IL-2, IL-4, γ-IFN) ■ iNOS/NO ■ growth factors ■ proliferation/activation of T-/B-lymphocytes ■ phagocytic activity ■ coagulation assays (aPTT, PT, TZ, Fxa) ■ matrix metalloproteases (MMPs: MMP1, MMP3, MMP9 etc.) ■ transcription factors (NF-κB, NF-AT, AP-1, OCT-1 and STATs) ■ signaling studies, MAPKs (ERK 1/2, p38, JKN/SAPK); PKCs; calcineurin dephosphorylation; cyclins activation, tyrosine kinases and the like ■ determination of phenotypes (CD determinations by FACS analysis): single and double staining ■ proliferation and cell cycle analyses by incorporation of 3[H]TdR/BrdU and/or FACS analysis: (a) mitogens (PHA, ConA, LPS); (b) TCR specific, CD3 or CD3/CD28 or with the superantigen SEB; (c) cell cycle analyses: progression to the S-phase and G2/M of the cell cycle

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- antigen specific proliferation in murine primed T cells, Th1 and Th2 profile by cytokine release
- allogenic T cell proliferation (mixed lymphocyte cultures)
- induction of antigen-specific antibody production *in vitro*
- natural Killer activity and CTL activity
- determination of cell death in primary cells by determining either necrosis or apoptosis (type I and II)
- signalling studies in primary T/B cells and in lymphoid tumor cells: calcium mobilisation, intracellular pH changes, and the like
- transfections in primary T cells and in lymphoid cell lines: functional studies of transcriptional regulation using a big panel of cellular and synthetic promoters cloned in front of the luciferase gene (NF-κB-Luc, AP-1-Luc, NF-AT-Luc, IL-2, TNF, IL-6, ICAM-1, CD69, cyclins, p21, Bax, PECAM, etc.)
- stably transfected cell lines with the luciferase gene driven by several inducible promoters regulated by PMA, IL-1 and TNFa-transcription factors (NF-κB, LTR-HIV)

7.2.2. Immunology / Inflammation: *in vivo* Services

Models

- Inflammatory Bowel Disease (IBD):
  A model for intestinal inflammation in Swiss mice comparable to Morbus Crohn and colon ulcerative diseases. The parameters to measure are morphological and histopathological.

- Septic Shock:
  Induced by LPS in Balb/c mice and the parameters to measure are survival and measurement of IL-1, TNFα, and IL-6 in serum. Transcription factors are also measured in isolated spleen cells of treated mice.

- Collagen Induced Arthritis:
  Morphological and histopathological criteria and phenotypical analysis of inflammatory infiltration in affected joints.

- Murine Models for Immunoglobulin Measurement:
  In vivo isotype switching, IgG and IgE

- Skin Allograft Rejection

- Delayed-Type Hypersensitive Reaction:
  Murine model for human allergic contact dermatitis.

7.3. Cancer / Oncology

The cancer / oncology services are offered in co-operation with VivaCell.

7.3.1. Cancer / Oncology: *in vitro* and *in vivo* Services

Models

- *In vitro* testing methods include proliferation inhibition assays using human tumor cell lines, as well as clonogenic assays with human tumor xenografts, human tumor cell lines, and hematopoetic stem cells. More than 350 solid tumor models have been established in serial passage as well as 20 permanent tumor cell lines (co-operation). In addition, a panel of 60 cell lines, commonly used in cancer research such as A549, MCF-7, Caco-2, HT29 or PC3 are also available. Cancer cell lines over-expressing the MDR1 gene are also available for drug research.

- *In vivo* assays include subcutaneous models, orthotopic models with human xenografts, murine tumour models (co-operation) and models of topic co-carcinogenesis inducing papilloma-like skin tumours in athymic mice.

Parameters

- proliferation, XTT assays, determination of DNA content by FACS analyses
- cytokines (e.g. IL-6), growth factors (e.g. EGF), etc.
- angiogenesis (e.g. VEGF, angiopoyetin I and II, nephrin, i.e.): *in vitro* models of angiogenesis using HUVECs and Matrigel Basement Membrane Matrix; *in vivo* models of angiogenesis by corneal neovascularization assays
- cell cycle analyses, cytotoxicity by measuring PI staining, determination of DNA fragmentation (apoptosis) by TUNEL
- transmembrane potential of the mitochondria, calcium and pH mobilisation
- reactive oxygen species (ROS)
- Fas clustering and signalling in type I and II cells for apoptosis
- caspases (3, 8, 6 and 9) activity by spectrofluorometry and western blots
- Bcl-2, Bcl-X, Bax, Bid and cytoplasmic cytochrome C by western blots
- apoptosis and cell cycle analyses in MDR cell lines
- phosphatidyl serine expression at the cell surface by Anexin V binding
- integrity of tubulin and actin by fluorescence microscopy
- *in vitro* assays for tubulin polimerization
- cyclins activity by IP and western blots
- activation of transcription factors

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- signalling studies MAPKs, IKK
- determination of telomerase activity in Hela cells by telomerase PCR ELISA
- determination of estrogenic or anti-estrogenic activity of phytoextracts using ER expression vectors (α y β subunits) and an specific ER responsiveness promoter driven the luciferase gene
- customized "knock out" cell lines for specific genes
- DNA microarrays for cancer-related genes in tumoral cell lines

Other specific cellular and molecular techniques are available upon request.

7.4. HIV-1 / AIDS

The HIV-1 / AIDS services are offered in co-operation with VivaCell.

7.4.1. HIV-1 / AIDS: *in vitro* Services

In vitro testing methods include cells lines specific for HIV-1 studies.

Models

- Jurkat cells stably transfected with the luciferase gene directed by the LTR-HIV promoter
- MT-2 cells for infection with the HIV-1 virus
- Tat-transfected cells, *in vitro* infection of primary T cells
- determination of IC-50 for anti-HIV drugs using an artificial proviral clone

Parameters

- inhibition of cell death by HIV-1 in MT-2 cells (MTT assays)
- determination of p24 antigen in culture supernatants of primary T cells infected with HIV-1
- determination of the transcriptional activity of the LTR-HIV promoter by Tat and by TNFα
- determinations of anti-HIV compounds targeting Tat/TAR interaction and RNA elongation
- determination of viral package in 293T cells

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Our Business
ADME in vitro / in vivo
Toxicology in vitro / in vivo
V79 Cell Battery™
Analytical Services
Service Packages
Partnered Services

7.5. Dermatology / Cosmetics

The dermatology / cosmetics services are offered in co-operation with VivaCell.

Highly specialised and reliable skin models for testing cosmetics and pharmaceutical compounds (phytopharma) are offered. Modern and standardised *in vitro* protocols according to ethical guidelines provide the advantage over *in vivo* traditional models. Selected *in vivo* models are also available.

7.5.1. Dermatology / Cosmetics: *in vitro* Services

Models

- animal skin organ cultures
- bovine and rabbit isolated cornea
- human normal keratinocyte monolayer cultures
- human normal fibroblast monolayer cultures
- red blood cells
- reconstituted human epidermis
- cell lines: keratinocytes (HaCaT, NCTC2544, ...), fibroblasts (MRC5, 3T3, ...), Madin Darby Canine Kidney (MDCK), etc.

Toxicity

Parameters

- cytotoxicity:
  MTT
  Neutral Red
- membrane damage:
  red blood cell lysis
  protein denaturation

Trans-Epithelial Permeability Effect

Parameters

- changes in permeability is measured as the leakage of fluorescein through a epithelial barrier:
  cornea
  skin
  confluent cell monolayer

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Phototoxicity or Protective Effects

Parameters

- changes in cell cultures or skin after a UV or visible light exposition:
  cytotoxicity and membrane damage
  oxygen radical production
  gluthation S-transferase activity of cells
  the complement photoactivation assay

Skin Firmness

Parameters

- measurement of adhesion molecules in cell cultures:
  keratinocytes
  fibroblasts

Extracellular Matrix Adhesion Assay

Parameters

- fluoresceinated cells are cultured on chambers coated with artificial extracellular matrix; fluorescence retained in such chambers after a short incubation period gives a measure of the capacity of cells to adhere in the presence of a test compound

Human Skin Fibroblast / Collagen Lattice Cytotoxicity Test

Parameters

- skin fibroblasts are incorporated into 3-d collagen lattices containing the test compounds; an inhibition of lattice contraction gives an indication of the deleterious effect of the compound under test

Healing and Migration Assay

Parameters

- the capacity to regenerate a "wound" in a cell monolayer culture or the capacity of cell to migrate across a porous membrane is measured

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7.6. Oral Care

The oral care services are offered in co-operation with VivaCell.

7.6.1. Oral Care: *in vitro* Services

Models

- human primary monocytes
- human normal fibroblast monolayer cultures (comparable to gingival fibroblasts)
- primary oral gingival epithelial cells (treated with pathogens)
- cell lines: fibroblasts (MRC5, 3T3), etc.
- VR1 transfected cells: activation of these cells through this nociceptive receptor could mimic the initial steps in neurogenic inflammation and it is suitable to test new analgesic compounds topically applied in oral care

Parameters

- inflammatory parameters: PGE2, LTB4, cytokines, etc.
- cytotoxicity: MTT, LDH, Neutral Red, etc.
- oxygen radical production
- cell proliferation
- angiogenesis
- healing and migration
- calcium mobilisation and cell death through agonists / antagonist of the VR1 in a heterolgous system

7.7. Partnered Services / Contacts / Network

- Physicochemical Properties
- Chemical Synthesis of Chiral and Non-Chiral Compounds
- NMR-Spectroscopy
- PAH-Analytics
- Phytopharmaka / Plant Extracts
- Nutrition
- Safety Pharmacology
- Inhalative Toxicology
- Phenotyping / Genotyping
- Clinical Studies

Please inquire for further partnered services.

7.8. Consulting

Both scientific and legal/regulatory expertises are necessary to establish streamlined test and regulatory submission strategies for your compounds.

GenPharmTox serves you as an experienced and accessible guide through the maze of scientific and governmental regulations.

Whether your company just needs advice, or more in-depth assistance, GenPharmTox is ready to provide custom resources to get the job done. We provide reliable and thorough assistance in navigating through the complex rules and regulations during drug discovery and drug development phases and a professional service for registration of your chemicals and drugs.

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