Institute of Cancer Therapeutics
Research Projects and Services
Forward from the Director

Prof Richard Morgan

Thank you for your interest in the Institute of Cancer Therapeutics (ICT), and for the opportunity to share some of our key research projects, which provide a range of exciting investment and/or partnership opportunities.

We also offer state of the art laboratories, including Ethical Tissue, a licenced tissue bank and the technical expertise to facilitate your research. Specialisms include synthetic and medicinal chemistry, pre-clinical pharmacology (including in vivo biology), molecular biology, drug metabolism and pharmacokinetics (DMPK), drug formulation, and proteomics.
Investment and Partnership Opportunities

Sponsored Research Projects (1-3 years)
- Industrial Studentships (3 years)
- EU Doctoral Training
- EU H2020 Partner
- RCUK Industry collaborative schemes
- Short term (3-6 month) contract research
- Pilot research projects (6-12 months)
- Knowledge Transfer Partnerships
- Consultancy
- Scientific Advisory

Further Information
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**FPRI antagonists**

**A First in Class Treatment for Glioma**

**The project**
Progression of many solid cancers is associated with the development of necrotic areas. In glioma and other cancers (prostate, breast, neuroblastoma), necrotic cells release peptides that activate the formylpeptide receptor-1 (FPR-1), which is highly expressed on the surface of tumour cells, but not on healthy cells.

Activation of FPR-1 is upstream of many drivers of cancer expansion, therefore a FPR-1 antagonist can affect multiple aspects of the disease progression (angiogenesis, invasion, proliferation and metastasis), without causing toxicity.

ICT12035 is a non-toxic small molecule FPR-1 antagonist which abrogates progression of glioma tumours in vitro and in vivo (sub-cutaneous model).

**The IP position**
A patent application for the use of ICT12035 in glioma has been prepared and is being filed by the Universities of Bradford/Leeds. Data collection to complete a second patent application, including novel proprietary FPR-1 antagonists, is underway.

**The opportunity**
To consolidate and expand the potential value of the IP, supporting FPR-1 antagonism as a key therapeutic target in cancer.

The next milestones are: (1) show clinical application of “radiotherapy+ICT12035” treatment for glioma in an orthotopic model (Leeds University collaboration). (2) complete the evaluation of novel FPR-1 small molecule antagonists in other solid tumours (prostate, breast, neuroblastoma).
TMEM92
A Novel Target and Marker in Prostate Cancer

The project
TMEM92 has a single transmembrane domain and is strongly expressed in a number of prostate cancer derived cell lines and primary prostate tumours, but not in normal prostate tumour or benign prostatic hyperplasia. We have produced mouse mAbs against the extracellular domain of the mature protein, revealing a distinct pattern of protein expression in cells strongly related to mitosis.

The IP position
the patent for the use of TMEM92 as a marker and therapeutic target in cancer has been granted in most territories.

The opportunity
To greatly increase the potential value of the IP studies to support its use as a target and/or marker in cancer. The next milestones would be to (1) demonstrate anti-tumour activity of the TMEM92 mAb in animal models and (2) establish whether TMEM92 in liquid biopsy is a diagnostic marker.

PROSTATE CANCER CELLS
NORMAL CELLS
Protease Activated Prodrugs for Tumour Selective Delivery

The project

Membrane-type matrix metalloproteinases (MT-MMPs) are known to be elevated in the majority of solid human tumours and to be central to tumor invasion and angiogenesis. MT-MMPs are absent or inactive in normal tissues. The objective has been to design inactive prodrugs that are converted to the active drug by selected MMPs specifically within the tumor microenvironment.

This technology has a very strong, proven track record of success. Previous studies using this approach with both azademethylcolchicine (ICT2588, awaiting clinical trial Q4 2017) and paclitaxel (ICT3205) were successful in targeting the drug release to the tumour, resulting in impressive 10-fold increases in tumour concentrations of warhead and impressive tumour responses with a single dose.

Ex vivo Assays are available in-house to assess activation of prodrugs in tumour tissue, and stability in normal tissues, which are highly predictive of in vivo studies.

The IP position

The IP surrounding ICT2588 and ICT3205 is licenced to Incanthera Ltd. We have now developed new tumour selective targeting technology and IP in Bradford using novel peptide sequences and seek investment to protect and develop further with highly potent warheads.

Ex vivo Assays are available in-house to assess activation of prodrugs in tumour tissue, and stability in normal tissues, which are highly predictive of in vivo studies.

The opportunity

There is potential to develop the highly promising taxane based prodrugs further in partnership with Incanthera Ltd. We also seek funding to develop novel agents to exploit this platform technology, to deliver highly potent warheads, and to develop a package with clinical potential. Specifically, investment is required to enable in vivo pharmacokinetics and efficacy studies of promising agents.
Ran GTP as a Novel Biomarker and a New Class of Cancer Therapeutics

The project
Ran GTP (Ran) is a Ras-related GTPase that is critical for mitosis, apoptosis and nucleocytoplasmic transport, and is overexpressed at both the mRNA and protein levels in breast and lung cell lines and tumours. We and others have shown that high Ran expression in tumours is associated with poor patient outcomes in breast, lung, ovarian and renal cell cancers. We have also demonstrated that Ran expression can predict breast and lung patient survival. In addition, we have shown that Ran overexpression plays a role in breast and lung cancer metastasis, highlighting a novel role for Ran in cancer progression.

We have produced small molecules and mouse and rat monoclonal antibody inhibitors of Ran with proven activity in vitro and in vivo.

The IP position
the patent for the use of RanGTP as a marker has been granted in most territories and a patent for its use as a therapeutic target in cancer has also been filed.

The opportunity
to greatly increase the potential value of the IP through studies to support its use as a target and/or marker in cancer. The next milestones would be to (1) demonstrate anti-tumour activity of the Ran mAb in animal models and (2) establish whether Ran in liquid biopsy is a diagnostic marker.

Ran inhibitor administration (G3, early & G4, late treatment) reduced tumour volume by 70-80% compared to untreated control mice (G2) and reduced metastasis by 80-90%.
CCR7 Antagonists for Prevention of Lymph Node Metastasis in Head & Neck Cancers

The project
Migration of cancer cells to the lymph nodes is a prelude to metastasis. We have shown that this process is initiated when tumour cells gain the expression of chemotactic receptor CCR7 enabling them to migrate towards the lymph nodes, guided by CCR7’s ligand CCL21.

We have discovered a series of novel small molecule CCR7 antagonists, which prevent migration and invasion of head & neck tumour cells in a number of in vitro models.

The IP position
ICT13069, and other members of this series, are the only known small molecule CCR7 antagonists.

A patent application is currently being prepared by the University of Bradford.

The opportunity
To consolidate and expand the potential value of the IP, supporting CCR7 antagonism as a key anti-metastatic target in cancer.

The next milestones are: (1) show clinical application of ICT13069 in an orthotopic model of head & neck cancer (Sheffield University collaboration). (2) complete the evaluation of ICT13069 and related novel CCR7 small molecule antagonists in a number of in vitro and in vivo models for other solid tumours (prostate, breast, pancreatic).

ICT13069 prevents the invasion of head & neck cells spheroids through collagen

INCREASED DRUG = DECREASED INVASION
Polysialyltransferase - a Novel Target for Neuroblastoma

The project
Polysialyltransferase (PolyST) catalyses the biosynthesis of polysialic acid (polySia) on the surface of neuroendocrine tumours, notably neuroblastoma (a paediatric cancer with high mortality and a desperate need for novel therapies). PolySia plays a key role in tumour migration, invasion and tumour dissemination and its expression is limited to tumour tissues post-embryogenesis. PolyST is thus a novel, validated target for anti-metastatic therapy.

We are engaged in a drug discovery programme to identify potent, selective polyST inhibitors. We have established a novel biochemical assay, a panel of cell lines (including naturally expressing, isogenic and knock-out cells) and cell-based functional assays to assess compound inhibition. Selective polyST inhibitors with low micromolar potency have been identified to-date. Ongoing experiments are focused on assessment of effects of compounds on polysialylation, migration and invasion in vitro. Systemic and orthotopic in vivo models have been identified. This project is currently supported by Yorkshire Cancer Research and The Wellcome Trust.

The IP position
Freedom to operate; clear IP landscape with molecules synthesised to-date. Patent yet to be filed.

The opportunity
To support the development of this highly novel approach to therapy; a first-in-class agent for high-risk neuroblastoma and other neuroendocrine tumours (e.g. SCLC). Specifically, to support the project towards achieving proof-of-concept in vivo. The next milestones would be (1) to identify a lead agent for progression to advanced DMPK studies and (2) to demonstrate efficacy in vivo (i.e. reduction in tumour dissemination).

PolySia expression in [a] neuroblastoma xenograft tissue and [b] neuroblastoma PDX tissue; [c] polySia (green) and GD2 (red) expression in clinical neuroblastoma bone marrow deposits
New Strategies for Integrin Antagonism

The project
The RGD binding integrins are involved in growth and metastasis of a number of cancers with significant unmet clinical need. Despite being the target of significant interest by the pharmaceutical industry, no effective integrin based anticancer agents have reached the clinic. We have developed a new integrin antagonist chemotype which can be applied to selective and polypharmacological antagonism or combination therapy involving integrin targeting.

IP position
Not yet patented. In vivo studies to support filing a composition of matter patent are in progress.

The opportunity
To progress selected compounds through lead optimisation.
CTC Isolation and Characterisation from Liquid Biopsies Using Microfluidics

The project
Microfluidic chips capable of separating circulating tumour cells (CTCs) from leukocytes without the use of antibodies are now available. The use of such devices to isolate CTCs will be assessed using blood samples from cancer patients (and healthy volunteers) whilst preserving serum for other bioassay studies. CTCs can be identified by immuno-fluorescent staining (e.g. DAPI, CK+, CD45-) and further characterised based on other markers such as MRPI&2, EpCAM or target proteins (MMPs, TMEM92 etc.).

The commercial position
The use of CTCs for prognosis and personalisation of therapy is increasingly being recognised. Such methods can be used to support clinical trials and identify biomarkers. Proprietary cell lines can also be developed from CTC.

The opportunity
Ethical Tissue is seeking to develop new services as part of its sustainable growth strategy, which has formed the basis of a recent MRC funding bid. Isolation, characterisation and long term culture of CTCs are part of the services that ET is seeking to develop from the use of liquid biopsies. Equally, such techniques should support the ongoing research activities of the ICT.

Development of HypoCSCell Reagent Kit for the Identification, Evaluation and Isolation of Cancer Stem Cells

The project
The project... Hypoxia in solid tumours provides a local milieu where cancer stem cells (CSCs) are protected and highly resistant to chemotherapy and radiation therapy and therefore positively correlate with the degree of tumour malignancy, as well as with a decreased overall survival rate of cancer patients. CSCs express high levels of aldehyde dehydrogenases (ALDHs), enzymes that participate in many physiologically important biosynthetic pathways. We are developing a kit, termed HypoCSCell, which can be commercialised and used to identify ALDH-expressing CSCs in the hypoxic tumour microenvironment.

The IP position
Research in Bradford has resulted in chemical probes, which show great potential for targeting ALDHs expressed in CSCs. The chemical probes are currently being linked to chromophores, which will enhance their fluorescent properties and hypoxia selectivity. No IP has been protected yet but data is being generated for patent application.

The opportunity
The hypoxic microenvironment of solid tumours contain CSCs that are resistant to conventional therapies and a likely cause of treatment failure. As a consequence, there is a clear market demand and opportunity for the development of HypoCSCell, a novel reagent kit, which identifies hypoxia-located CSCs. Commercialisation of HypoCSCell will enable better interpretation of the heterogeneity of solid tumours and allow resistance mechanisms present in hypoxic CSCs to be profiled. Ultimately, such information will lead to better choice of therapeutics to treat solid tumours and lead to an increase in patient survival rates. Funding is sought to support proof-of-concept studies to identify the best chemical probe, which can secure the IP and be used as the active reagent of the HypoCSCell kit.

Figure 1 HypoCSCell, a reagent kit to identify CSC residing in the hypoxic niche of solid tumours. (A) Solid tumours contain hypoxic niches and CSC residing in these low-oxygen pockets are contributing to treatment failure (A). A CSC-targeting fluorescent reagent kit (HypoCSCell, B) can be used to identify these malignant cells which can be isolated using fluorescence-activated cell sorting (FACS) equipment (C).
Cytochromes P450 as Targets for Tumour-selective Activation of Ultrapotent Chemotoxins

**Aim**
The project...Cytochromes P450 (CYPs) are a superfamily of mixed function oxidases of which CYP1-4 subfamily members are unique in their ability to oxidise drugs. CYP1A1, 1B1 and 2W1 are expressed in many human tumour types to a high frequency and we have developed therapeutics based on the duocarmycin scaffold that are selectively activated by these CYPs (Figure 1).

**The IP position**
The patent covering compound structures based on the duocarmycin pharmacophore to treat CYP-expressing cancers has been granted in most territories while new IP will be sought on second generation duocarmycin analogues.

**The opportunity**
To greatly increase the potential value of the IP studies to support the use of duocarmycin prodrugs as novel agents to treat patients with CYP-expressing tumours (incl. bladder, breast, colon, glioma and head and neck cancers). The research is funded by Yorkshire Cancer Research and background IP is licensed out to Incanthera (www.incanthera.com/). The next milestones is to demonstrate anticancer activity of lead compounds in a number of in vivo models and with investment foreground IP can be acquired.

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**Figure 1** CYP-bioactivated tumour-selective duocarmycin prodrugs. (A) Extra-hepatic CYP isoforms overexpressed in cancer tissues, (B) Proposed mechanism of bioactivation of duocarmycin bioprecursor (compound 1) and (C) in vivo investigation showing significant growth delay in CYP2W1-expressing tumours (left graph) with no effect on tumours not expressing CYP2W1 (right graph).
Specialist Capabilities

- Complex Synthesis
- Formulation Strategies for Poorly Soluble Compounds
- Preclinical Drug Screening and Evaluation
- DMPK and Clinical Pharmacology
- Ethical Tissue
- Proteomics
Proteomics

“Proteomics is the examination of a complete set of proteins synthesized by a cell under a given set of physiological or developmental conditions”

MARC WILKINS, 1994

Applications
Identification and validation of targets for drug development
Identification and validation of biomarkers
Pharmacoproteomics and toxicoproteomics
Exploring cellular and molecular mechanisms
Biopharmaceuticals characterisation

Samples
Cell lines – 2D and 3D models
Secretomes
Tissues/biopsies
Biofluids
Forensics

Quantitative proteomics
Metabolic labelling (SILAC), Isobaric tags (iTRAQ, TMT)
Parallel reaction monitoring
Label-free quantification

Specific strategies
Phosphoproteomics
PTM characterisation
Targeted proteomics – chemical proteomics

Data processing/Statistical analysis
Cluster analysis
Principal component analysis
Volcano plots
Correlation coefficients

Bioinformatics analysis
Gene ontology profiling
Protein-protein interactions
Metabolic pathway analysis
Function and mechanistic significance

Proteomics is the examination of a complete set of proteins synthesized by a cell under a given set of physiological or developmental conditions

MARC WILKINS, 1994

Protein Fragments
Peptides
Protease
MS/MS

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Protein
Peptides
Fragments
Formulation Strategies for Poorly Soluble Compounds

The project
Many of the small molecule anticancer drugs developed in the ICT have poor aqueous solubility. This can affect the results of in vitro assays and limit the ability to perform in vivo tumour regression and pharmacokinetic studies. Laboratory scale synthesis is also limited (100-200 mg typically), consequently little material is available for drug solubility assessment. Small scale formulation development has been carried out with a range of in-house compounds (ICT2588, ICT2700, ICT2035, ICT9119) using as little as 30 mg of compound and a variety of formulation approaches.

The commercial position
Lack of early formulation development has also resulted in expensive commercial formulation development and delayed regulatory safety studies when taking drugs forward towards clinical trials. Formulation development can often form part of the IP portfolio associated with drug development projects.

The opportunity
1) Work with colleagues in Pharmacy and Chemistry to develop additional formulation approaches, e.g. microfluidics and novel polymers. 2) Continue to use this approach for in-house development to add value to drug development programmes. 3) Provide similar service to external organisations conducting in vivo work at the ICT, first such contract completed for Incanthera (Es5).

In-house formulation approaches
Preclinical Drug Screening and Evaluation

The Preclinical Screening team at the ICT has over 30 years experience in offering in vitro and in vivo evaluation of novel anticancer therapeutics.

Experience of evaluating:
- Small molecule chemotherapeutics
- Antibodies
- Peptides
- Nanoparticles
- Drug delivery systems
- Gene delivery

In vitro and in vivo models for a range of cancer types including:
- Breast
- Prostate
- Lung
- Colon
- Melanoma
- Renal
- Pancreatic
- Head & neck
- Ovarian
- Bladder

In vitro evaluation
- Cell survival & apoptosis: tetrazolium-based cytotoxicity assays; clonogenic assay; various apoptosis assays including flow cytometry and microscopy based evaluations.
- Cell migration: scratch assay; Boyden transwell assay; agarose spot assay
- Cell invasion: Boyden transwell matrigel assay; 3D spheroid invasion assay
- Cell cycle: flow cytometry-based analysis; cell cycle marker immunodetection
- Angiogenesis: tube formation assay

In vivo evaluation
- Tumorigenicity: sc or site-specific implantation; monitoring of tumour growth; histopathology
- Determination of Maximum Tolerated Dose: iv, ip, po, sc or it; single/multiple dosing
- Hollow Fibre Assay: intermediate assay between in vitro & solid tumour efficacy; up to 3 cell lines; fibres implanted ip and sc on day 0; treat days 3-6; remove fibres day 7 and assess for effect using MTT assay; also adapted for pharmacodynamic (PD) analysis
- Efficacy in sc transplantation models: xenograft or allograft; iv, ip, po, sc or it; single/multiple dosing; use of satellite groups to analyse PD effects
- Orthotopic or site-specific experimental tumour models: please enquire
- Provision of tumour, tissue and blood/plasma samples for pharmacokinetic/PD analyses
- Non-invasive optical imaging: bioluminescence or fluorescence
DMPK and Clinical Pharmacology

The Institute has an experienced Drug Metabolism and Pharmacokinetic (DMPK) Team, with an efficient and well resourced analytical set-up. The DMPK team plays a central role in the drug discovery process within the ICT and works closely with other members of the Institute in studies both at preclinical and clinical stages. ADME, tissue distribution, and studies with recombinant enzymes are routinely undertaken. Previous studies have included the analysis of complex metabolic profiles and the development of analytical methods for the PK analysis of highly reactive and potent molecules in the picomolar range.

Pharmacokinetic Analysis

Non-clinical samples:
- Analytical method development with HPLC/UPLC and LC MS/MS
- Chiral analysis with HPLC and LC MS/MS
- Full pharmacokinetic profiles of drug and metabolites including tissue distribution (iv, ip, po)/ADME/bioavailability
- In vitro drug metabolism/metabolite identification/drug stability

Protein binding
- Analysis of small molecule biomarkers by LC MS/MS
- Pharmacodynamic markers in preclinical tissue samples.
- Cell uptake and tissue distribution of naturally fluorescent/fluorescently tagged agents by confocal microscopy

Clinical samples:
- Good Clinical Practice (GCP) laboratories in line with the guidelines from MHRA
- Validated analytical method development to GCP
- Full clinical pharmacokinetic reports
- Analysis and storage of clinical tissue/plasma samples for drug/metabolites and pharmacodynamic endpoints.
Ethical Tissue

Ethical Tissue is an ethically approved human research tissue bank, licensed by the Human Tissue Authority (HTA), housed within the Institute of Cancer Therapeutics (ICT) at the University of Bradford.

Ethical Tissue provides a wide range of human tissue, cells and fluid samples for biomedical research groups in academia, industry, and the health service. They are one of a few tissue banks who can provide tissues from a wide range of diseases together with normal comparators. In addition, our streamlined approval process means that researchers can receive ethical approval for sample requests within 4 weeks, without the need to obtain individual National Research Ethics Services (NRES) approval.

Tissues can be accessed in a number of formats:
- Fresh tissue
- Snap frozen tissue
- FFPE pathology blocks
- OCT embedded tissue and microscopy sections

Isolated primary cells
Subcellular fractions
Protein extracts

Tissue data is collected, stored and supplied by us. All samples are anonymously coded and personal data is protected. A minimal dataset is supplied with all tissue samples which may include age, gender, ethnicity and medical history.

The agreed collection protocols for each tissue cover their acquisition and transportation to the laboratory in order to preserve the integrity of the tissue samples. Pre-processing of samples can be arranged immediately after collection with temporary storage in Ethical Tissue before transfer to the researcher.

All samples are logged onto a central database in order to ensure traceability of all samples using the unique code assigned to each donation. Personal donor information is never issued and is kept under strict security and confidentiality.