University of BRISTOL

University of Bristol Cancer Research Fund (UCRF)

2020/21 Report

July 2021

Introduction

The University of Bristol Cancer Research Fund (UCRF) supports a range of cancer research from across the University. Importantly, your generosity helps start truly innovative projects and exciting new collaborations across different disciplines at the University of Bristol, all working towards the prevention and treatment of cancer. Our aim is to help fund early-career researchers and/or pilot studies, which are critical when developing research ideas. Without the evidence gathered at this early stage it is impossible to attract additional funding for larger and more ambitious studies. UCRF funding comes entirely from donations from the public, ranging from individual donations to charity fundraising events.

After the delays and frustrations of the first lockdown, we were really pleased to be able to fund 10 innovative, cutting edge projects this year. Thanks to your generous donations to the UCRF, the total investment in research this year was £39,699. These projects reflect the range of ground-breaking research taking place at the University and include a project using dog bone tumours as a model to develop new therapies for human osteosarcoma (page 3) and a study looking at the psychological and emotional effects of diagnosis, treatment, and prognosis of ovarian cancer (page 4). The details of all the studies funded in 2020/2021 are included in this report.

To illustrate how important the UCRF funding is, Dr Sabine Hauert and colleagues have received a UKRI Trustworthy Autonomous Systems HUB grant of £150,000 for their highly innovative project investigating an entirely new approach to study how tumours grow. This application was based on the data generated from a UCRF 2019/2020 grant, and I am sure this is only the beginning for this ground-breaking research.

In summary, the UCRF fund continues to be hugely influential in supporting early career researchers and large grant applications and I would like to thank you once again, for your generosity in supporting Cancer Research at Bristol.

Prof Ann Williams
Chair for the UCRF

Researcher	Dr Grace Edmunds, Bristol Veterinary School (BVS)
Study	Establishing a canine tissue repository to enable translational research in osteosarcoma
Total award	£4,327

Osteosarcoma is a painful and debilitating cancer, primarily affecting adolescents. Although it is considered rare, 90% of patients present with metastatic disease (where the cancer spreads from its original site to other parts of the body), and survival in these cases is just 20% at 5 years. Research to devise new therapies for osteosarcoma is hampered by the rarity of patient samples, and the enormous diversity in its tumour genetics, which make a “one size fits all” solution challenging to define.

Osteosarcoma is 27 times more common in dogs than in humans, and particular breeds such as the Rottweiler are at higher risk, because inbreeding of dogs causes risk-associated genetic variants to become concentrated within the breed. Canine osteosarcoma has highly similar genetics, microenvironment and clinical course to the human disease, and dog bone tumours therefore represent an excellent and abundant model with which to develop new therapies for human osteosarcoma.

As a solid tumour grows, the levels of oxygen inside the tumour can drop, resulting in a condition known as hypoxia. This sets off signalling within the cells (hypoxia-inducible signalling) which helps the tumour cells survive in these low oxygen conditions. In a collaboration between BVS, Cardiff University, Minnesota Veterinary College, University College London and the Royal Vet College/VetCompass, a multi-disciplinary programme of work has been devised that will utilise Rottweiler tumour samples to determine the extent to which hypoxia affects progression and prognosis in osteosarcoma. Although drugs blocking hypoxia-inducible signalling are licensed and could be repurposed for tumour-immunotherapy, their potential for use in refractory cancers such as osteosarcoma has not been explored.

Central to this work is the generation of a canine osteosarcoma tissue repository at Bristol Veterinary School, which has been funded by the UCRF. Although the onset of tissue collection was delayed owing to COVID-19, our time during the pandemic was used to publish the largest demographic risk-factor analysis in canine osteosarcoma to-date (1). Furthermore, we produced a £2 million BBSRC grant application which aims to expand the proposed canine tissue repository to multiple other cancers and disease conditions. The first samples have now been collected, and we look forward to reporting the results of preliminary studies at the end of this year. Our work on this project to-date has been featured in the Irish Veterinary Times, the Mail Online, and the PetBuzz podcast.

1. Edmunds GL, Smalley MJ, Beck S, Errington RJ, Gould S, Winter H, et al. Dog breeds and body conformations with predisposition to osteosarcoma in the UK: a case-control study. Canine Med Genet. 2021;8(1):2.

Researcher	Dr Alyson Huntley, Bristol Medical School (PHS)
Study	Women and Cancer: supporting treatment adherence and survivorship
Total award	£4,886

I have a special interest in self-care and self-management approaches for women with cancer. Gaining support from the UCRF has enabled me to work with women with cancer, their health professionals and relevant charities. During the past year I have conducted review projects looking at the personal experiences of women with cancer: these include a study of women with breast cancer undergoing chemotherapy, an overview of the use of mistletoe in cancer research (we are about to publish results from a feasibility study in this area, previously supported by the UCRF) and currently I am conducting a review of the psychological and emotional effects of diagnosis, treatment, and prognosis of ovarian cancer. I work with health professionals and patients both in the community and hospital care across Bristol.

This funding has allowed me to apply for projects from European funding schemes which although were not successful, the concept was highly praised. Therefore my plan for 2021/2022 is to refine the application and apply for further funding to support the research prioritisation project with women diagnosed with ovarian cancer. These applications would not be possible without the support from the UCRF, for which I am very grateful.

Researcher	Mr Adam Chambers, School of Cellular and Molecular Medicine and BHBW NHS Foundation Trust
Study	Determining the response of rectal cancers to neoadjuvant chemoradiation using circulating tumour DNA from liquid biopsies
Total award	£4,017

I want to take this opportunity to thank the University Cancer Research Fund for this funding. It has enabled the analysis of next generation sequencing data (a means of studying the genetic information of cancer cells) circulating in the blood samples taken from patients undergoing chemoradiation for locally advanced rectal cancer. Patients who undergo this type of therapy have a varied response and understanding how a patient has responded is one of the current challenges in the management of this disease.

We aim to use circulating tumour DNA (fragments of DNA derived from the tumour that can be isolated in blood samples) to determine whether the chemoradiation has been successful before the patient undergoes surgical removal of their tumour. The funding awarded has been used to access specialist bioinformatics help for the setup of the data analysis pipeline. This work is ongoing, having been impacted by the coronavirus pandemic due to clinical commitments and as such has been slightly delayed. It is anticipated that these pilot data will lead to a larger prospective study, which would have not been possible without the help from the UCRF.

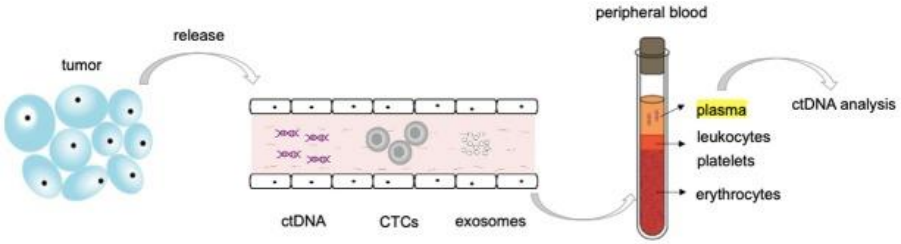


Figure 1. Using circulating tumour DNA to determine response to chemoradiotherapy.

Researcher	Dr Christin Hoffman, Bristol Medical School
Study	Factors influencing the adoption of surgical innovation: systematic review of frameworks of innovation diffusion
Total award	£1,100

Innovation is important to advance surgical care for cancer patients. Unlike the introduction of new pharmaceuticals, new surgical procedures are often not as rigorously tested before they are used in the clinic. Surgeons' decision to adopt new procedures (e.g. robotic techniques) or devices (e.g. novel surgical implants) are not based on principles of best available evidence, but are motivated by other factors (e.g. enthusiasm, perceived patient benefit). This can cause delayed uptake of effective innovations or can lead to disinvestment in harmful ones, as highlighted in recent high-profile investigations (Cumberlege report [1]). This suggests that the motivational factors underpinning surgeons' decisions to adopt surgical innovations are not fully understood.

Our study aimed to systematically review the literature from these investigations to identify factors and theoretical frameworks that may explain surgeons' adoption behaviour. A summary of such factors and frameworks relevant to surgical innovation will help map interventions (i.e. treatments and procedures) that optimise safe introduction of surgical innovation. With support of UCRF funding, this work has laid the foundations for the development of a Wellcome Trust fellowship application which aims to continue this work. Continued work will seek to map facilitators and barriers in order to identify and prioritise targets for intervention development that aim to address the insufficient use of formal protocols when adopting innovations. Ultimately, a change in behaviour towards an increased uptake of formal research protocols will accelerate standardised and safe introduction of innovative care for cancer patients.

1. *Cumberlege J. First do no harm: the report of the Independent Medicines and Medical Devices Safety Review. 2020 Jul 8.* https://www.immdsreview.org.uk/Report.html

Dr James Yarmolinsky *Dr Emma Vincent*

Researchers	Dr James Yarmolinsky and Dr Emma Vincent, Bristol Medical School (PHS)/School of Cellular and Molecular Medicine
Study	Exploring the mechanism underpinning the protective effect of statins in ovarian cancer
Total award	£5,000

Ovarian cancer is the second most common gynaecological cancer in the UK and accounts for more deaths than all other gynaecological cancers combined. There is currently no established screening test for this disease so identification of novel strategies for the prevention of ovarian cancer could be an important vehicle for disease control.

Statins are commonly prescribed low-density lipoprotein (LDL) cholesterol lowering medications for the prevention and management of cardiovascular disease. Meta-analyses of observational epidemiological studies have reported that women who use statins have a lower risk of ovarian cancer as compared to non-users. We have also recently shown that women who have genetic variants that mimic the LDL cholesterol lowering effect of statins have a lower risk of ovarian cancer. While these findings suggest that statins could be used as a preventive therapy for ovarian cancer, there is currently little understanding of the specific molecular mechanism(s) that may be driving this protective effect.

To explore the molecular mechanisms, we are carrying out an experiment to look at how statins impact protein expression. To do this we are using a technique called mass spectrometry, which measures the levels of all detectable proteins expressed within the cell (also known as proteomics). Due to the pandemic, we are a little behind with our lab work schedule. So far, we have completed the preliminary experiments needed to determine the optimal experimental conditions required for the proteomics experiments. We have used these experiments to determine which ovarian cancer cell lines to use and which dose of statin to use. Over the next couple of months we will run and analyse the proteomics data. We are excited to see how protein expression is changed upon statin treatment and to use these data to guide future population health science-based approaches.

Researcher	Dr Bethan Lloyd-Lewis, School of Cellular and Molecular Medicine
Study	Investigating the impact of breast cancer-associated developmental exposures on breast epithelial cell fate
Total award	£4,800

Breast cancer is the most common cancer in the UK, with an estimated 4,000 new cases diagnosed every month. The risk of developing breast cancer varies greatly throughout a woman's lifetime. Whilst an early puberty is associated with an increased risk of developing breast cancer, pregnancy before the age of 20 has a protective effect. Breast development, therefore, represents a crucial period in determining a woman's risk of breast cancer. My research is focused on discovering the relationship between exposures that impact breast development and breast cancer risk.

A recent genetic epidemiology study by our collaborators at the University of Bristol MRC-Integrated Epidemiology Unit suggested that higher obesity during childhood may be protective against breast cancer. Using genetic epidemiology techniques, we identified several growth factors and hormones affected by a high BMI that might mediate the protective effect of early life adiposity on later life breast cancer. The overall goal of this project funded by the UCRF was to grow mammary (breast) epithelial cells in 3D in a dish, and treat them with these factors to investigate their effect on the growth and cellular composition of these 'mini-glands'.

With the support of the UCRF we were able to purchase the reagents required to establish mammary epithelial cells in 3D cultures, and the factors identified by our genetic epidemiology analysis. Examples of 'mini-glands' growing in a dish and imaged using different microscopy methods are shown in Figure 1. Although the COVID-19 pandemic has restricted our lab activity, we have optimised our culture conditions and will soon start characterising the impact of the identified factors on the growth and morphology of mini-glands (A), and on the quantity and location of the major mammary epithelial cell types (basal and luminal) that make up the breast epithelium (B). Moving forward, our goal will be to also investigate the impact of these factors on the growth and composition of 'mini-glands' established from breast cells obtained from the mastectomies of women with a genetic predisposition to developing breast cancer (such as women who carry mutated BRCA1/2 genes).

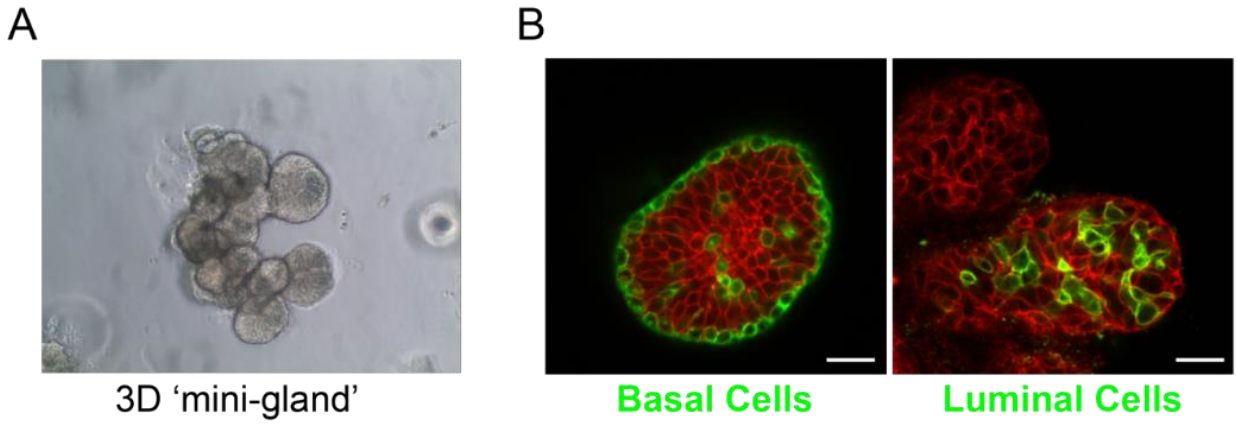
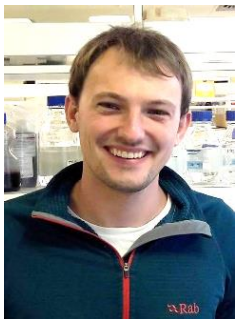


Figure 1. (A) Representative image of a 'mini-gland' established from mammary (breast) epithelial cells grown in 3D culture for 15 days. (B) Fluorescence images of 'mini-glands' where different mammary epithelial cell types are labelled with a green fluorescent protein.

The left image shows the localisation and quantity of basal mammary epithelial cells, which are mostly confined to the outer layer of the structure. The right image shows the localisation and quantity of a sub-set of luminal mammary epithelial cells residing in the inner layer of 'mini-glands'. Most breast cancer subtypes are believed to develop from luminal mammary cell types.

Dr Gareth Jones



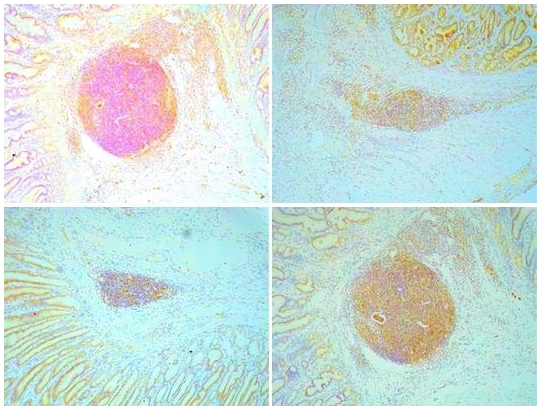
Dr David Hill

Researchers	Dr Gareth Jones & Dr David Hill, School of Cellular & Molecular Medicine
Study	Identifying mechanisms that promote tertiary lymphoid structure activity to enhance the ability of the immune system to destroy tumour cells
Total award	£4,979

The tumour microenvironment (TME: normal cells, molecules, and blood vessels that surround and feed the tumour cells) can suppress the body's immune response, allowing tumour cells to evade detection. Immunotherapies for cancer treatment aim to overcome the immunosuppressive tumour microenvironment and enhance adaptive immune responses to tumour antigens. For example, immune checkpoints are pathways normally involved in stopping the immune system from attacking healthy cells, but which tumours can use to evade immune destruction. Blocking the immune checkpoint proteins has been shown to be an effective way to increase the ability of an individual's immune system to recognise and destroy cancer cells in the body (immunotherapy).

In some tumours, immune cells can form organised clusters called tertiary lymphoid structures (TLS), that can support the ability of the immune system to destroy the tumour cells (anti-tumour immunity). Indeed, TLS presence is linked to improved metastasis- and disease-free survival in many cancers (e.g. breast, gastric, non-small cell lung carcinoma, sarcoma). Recently, TLS have also been linked with improved responses to immunotherapy. This highlights the potential of inducing TLS in tumours and combining approaches that promote TLS activity with immune checkpoint blockade as exciting prospects for novel treatment strategies.

Studying the relationship between TLS and the tumour has been hampered by the lack of available cancer models that feature TLS formation. However, our group recently discovered that TLS develop alongside tumours in a clinically relevant model of gastric cancer. This project uses this unique model in combination with deep sequencing methods (RNA-sequencing) and immunohistochemistry to identify mechanisms involved in TLS development and activity.



Despite the COVID-19 pandemic, we have managed to generate and import tumour samples required for the study from our collaborators in Melbourne, Australia. We find that tumour-associated TLS contain a high number of cells expressing the co-inhibitory receptors CTLA4, LAG3, TIGIT and PD-1 (Figure 1). We will soon perform RNA sequencing of the gastric tumour and tissue to identify gene signatures and molecular pathways that may present opportunities to modulate TLS as an approach to enhance cancer treatment.

Figure 1. Coinhibitory receptors are expressed at tumour-associated TLS in inflammation-associated gastric cancer. Immunohistochemistry for co-inhibitory receptors. TLS are visualised as dense clusters of cells (stained in blue). Staining for co-inhibitory receptors, seen in brown, includes: Top left - CTLA4; Top right - LAG3; Bottom left - PD-1; Bottom right - TIGIT.

Researcher	Dr David Morgan, School of Cellular and Molecular Medicine
Study	Analyses of transcriptome changes in regulatory T lymphocytes during tumour escape from PD-1 checkpoint blockade
Total award	£3,500

The aim of this work is to characterise the T-lymphocytes which reside in the tumour microenvironment and help tumour cells escape immune detection. This work will help identify those patients who will most benefit from the use of immune checkpoint inhibitors (biomarkers) and find new ways to improve the efficacy of immunotherapy (increase response and/or prevent relapse).

Background: The tumour microenvironment can help prevent tumour cells being recognised by the body's immune system. As described in Dr Jones and Dr Hill's study, this can result from increasing immune checkpoints (pathways which tumours can use to evade immune destruction), the presence of soluble inhibitory factors, and the presence of T-cells that can suppress the immune response. PD-1 is a major immune checkpoint. However, a proportion of tumours fail to respond or relapse following inhibition of PD-1. Recent work within our laboratory has identified subsets of regulatory tumour-infiltrating T-cells (T_{regs}), expressing multiple coinhibitory and costimulatory receptors, that are enriched in tumours that fail to respond to PD-1 blockade (Figure 1). We hypothesise that expansion of T_{regs} populations may contribute to resistance to checkpoint blockade in these tumours. The mechanism by which T_{regs} exert suppression within these tumours is not yet known, however insights into transcriptional signatures (the regulation of specific genes) occurring in these cell populations following PD-1 blockade may reveal potential biomarkers for treatment.

In October 2020, UCRF funds were awarded to enable us to perform transcriptomic analyses of T_{regs} following *in vivo* anti-PD-1 checkpoint blockade therapy. However, the *in vivo* work, which is vital to this project, was affected by the pandemic. Fortunately, we are now back on track, and we anticipate completing these studies over the summer of 2021. It is anticipated that these transcriptomics analyses will identify gene signatures within T_{reg} populations underlying the functional changes that we have identified by flow cytometry (a method used for identifying different physical and chemical characteristics of cells). This will lead to high-impact, peer-reviewed publications and will be highly informative within this exciting field of cancer immunology.

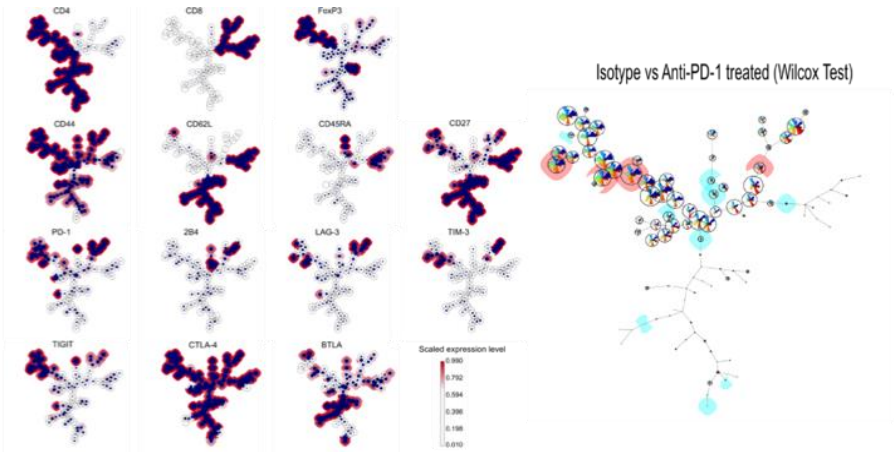


Figure 1. Dimensionality-reduction visualisation of the immune landscape of tumour-infiltrating T_{regs} & lymph node (LN)-derived T_{regs} in the context of co-inhibitory checkpoint (CIR) expression. Results of the FlowSOM algorithm are plotted in a minimum spanning tree. Live lymphocyte populations are plotted as clusters of "nodes". Mean expression profiles are shown for each node in a central star chart, where the size of each section represents the fluorescence intensity within each acquisition channel after scaling. Node size is relative to the number of cells being represented. Background colour indicates the automated metaclustering result, which identifies how node clusters lie within sub-populations.

Dr Natasha Clayton

Professor Anne Ridley

Researchers	Professor Anne Ridley and Dr Natasha Clayton, School of Cellular and Molecular Medicine
Study	Investigating the regulation and function of the atypical Rho GTPases RhoU and RhoV in T-cell acute lymphoblastic leukaemia (T-ALL) and prostate cancer
Total award	£5,090

T-cell acute lymphoblastic leukaemia (T-ALL) is a type of leukaemia which is aggressive and progresses quickly. The migration of T-ALL cells across the endothelial cell layer that lines blood vessels is a key step in T-ALL metastasis. In the laboratory, we have shown that the ability of T-ALL cells to efficiently cross endothelial cell layers is dependent on the protein RhoU, and that RhoU levels are elevated in T-ALL patient samples. Determining how RhoU activity is regulated is therefore important for understanding the mechanisms underpinning T-ALL cell metastasis.

Our results indicate that RhoU is able to self-associate (form aggregates) in cells, and that this causes T-ALL cells to adopt a more migratory shape. This suggests that disrupting RhoU self-association might reduce the metastatic potential of T-ALL cells. The UCRF award has enabled us to study RhoU self-association in greater detail, through funding **f**luorescence **l**ifetime **i**maging **m**icroscopy (FLIM) experiments that have allowed us to visualise precisely where in the cell these RhoU complexes form. We are now using FLIM to quantify how RhoU self-association is influenced by levels of other proteins already known to interact with RhoU.

We are also currently testing whether purified RhoU can self-associate in a test tube, or if other cellular proteins are required to link RhoU molecules together. Using UCRF funding, we have successfully purified human RhoU from an insect cell expression system and we will now use **m**ulti-**a**ngle **l**aser **l**ight **s**cattering (MALLS) to determine the size of any RhoU complexes in the samples.

Due to COVID-19 restrictions and technical issues with equipment in the Wolfson Imaging Facility, FLIM experiments could not be started until January 2021. Access to equipment required for RhoU purification has also been restricted due to COVID-19-associated capacity limits in our collaborator's lab.

Researcher	Dr Natalie Blencowe, Bristol Medical School (PHS)
Study	Treating patients with early oesophageal cancer: an evaluation of the role of endoscopic and surgical therapies
Total award	£2,000
Delayed as Natalie is currently on maternity leave.	

University of BRISTOL

Thank you very much for supporting the University of Bristol Cancer Research Fund.