Institution: University of Kent

Unit of Assessment: 3: Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Facilitating New Research Avenues and Transforming Clinical Screening and Teaching Programmes through the Discovery of a Novel Immunosuppressive Pathway in Acute Myeloid Leukaemia

Period when the underpinning research was undertaken: 2013-2020

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Vadim Sumbayev	Senior Lecturer	2006-present
Yuri Ushkaryov	Professor Honorary Professor	2012-2020 2020-present

Period when the claimed impact occurred: 2013-2020

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact (indicative maximum 100 words)

Researchers at the University of Kent have discovered a fundamental biochemical pathway that blood/bone marrow cancer cells use to escape immune surveillance. Some components of this pathway expressed only by cancerous and not by healthy cells can be used to diagnose and treat this high mortality disease. Three research collaborators – European Commission Joint Research Centre (Italy), Diamond Light Source Ltd (UK), and the University of Oldenburg (Germany) – have altered their research priorities and strategies, and initiated new projects, as a result the Kent team's discovery. Two hospitals – the University Medical Centre Hamburg-Eppendorf (Germany) and the University Hospital of Bern (Switzerland) – have made changes to their medical screening procedures and are now screening patients' samples for components of the new pathway (including the proteins latrophilin-1, galectin-3, and Tim3). The Kent team's discovery has been included in teaching programmes for medical and biomedicine students (undergraduate and postgraduate) at the University of Basel, (Switzerland), the University Hospital, Bern, and the University of Oldenburg.

2. Underpinning research (indicative maximum 500 words)

Background

Acute myeloid leukaemia (AML) is a blood/bone marrow cancer that affects over 250,000 people each year worldwide, including a large number of children and the elderly. AML is often fatal, with high mortality rates being associated, in part, with age at onset and ineffective treatments that have potentially harmful side effects. Current treatments include aggressive cytotoxic chemotherapy and stem cell transplantation. New tests to enable early diagnosis, and less toxic and non-invasive approaches to the treatment of acute myeloid leukaemia, are urgently needed. The University of Kent has carried out research aimed at addressing this challenge.

University of Kent research into acute myeloid leukaemia

Since 2013, Dr Vadim Sumbayev and Professor Yuri A. Ushkaryov, at the University of Kent's Medway School of Pharmacy, together with Dr Bernhard Gibbs (Kent 2006-17, University of Oldenburg since 2017), have carried out fundamental research into the underlying molecular mechanisms that enable AML cells to escape immunosurveillance (attack from the human body's own anti-cancer immune defences) **[R1-R5]**, and have developed novel strategies for the diagnosis and treatment of AML **[R6, P1, G1]**.

The Kent team have discovered that AML cells possess an immunosuppressive biochemical pathway **[R1, R2]**, which helps them to avoid the immune system **[R1-R3]**, and have shown that this does not exist in healthy blood cells, but develops as a result of malignant transformation **[R1, R2]**. Between 2017 and 2018, Sumbayev and colleagues showed that AML cells ectopically express a set of defence proteins and possess a secretion-signalling mechanism that is absent in healthy leukocytes **[R1, R2]**. In collaboration with Diamond Light Source (the UK's national synchrotron facility), the Kent team have used an innovative synchrotron-based approach to probe the interactions of proteins involved in AML immune escape. The first step in this mechanism employs a cell-surface receptor, latrophilin-1 (LPHN1) **[R1, R4]**. LPHN1 is an adhesion G-protein-coupled receptor that is normally present in neurons, but can also be ectopically produced by cancer cells **[R4]**. When latrophilin-1 is activated, it stimulates the release of the Tim-3/galectin-9 complex **[R1]**, which then interacts with immune defence cells, killing cytotoxic T lymphocytes and inhibiting the activity of natural killer lymphoid cells, thus protecting AML cells from being removed from the blood stream **[R1-R6]**.

Sumbayev and colleagues' discovery has led to a fundamental non-incremental breakthrough in the understanding of the pathophysiology of AML, which has opened up new avenues for diagnosis and treatment. (A short film describing the ways in which the Kent team are applying their findings has been produced.) Latrophilin-1 is being developed as a novel biomarker of acute myeloid leukaemia, which, unlike other known AML biomarkers, is expressed only in malignant, but not healthy, cells. This discovery has been registered in an international patent **[P1]** and has received worldwide media coverage. Furthermore, using pioneering technology based on the generation of functional nanomaterial in the form of gold nanoparticle conjugates **[R6]**, the Kent team have now developed and successfully tested a pilot leukaemia diagnosis kit, which will form the basis for designing a commercial kit for simple, robust, and rapid diagnosis of AML. The Kent researchers are also working towards the development of an anti-AML immunotherapy treatment. This approach aims to target and remove critical components of the pathway, thereby potentially enabling the patient's immune defences to eliminate AML cells **[R7]**. In summary, the Kent team's work represents a significant step towards a test for early diagnosis of AML and the development of new treatments of this high mortality disease.

3. References to the research (indicative maximum of six references)

[R1] Gonçalves, Silva I., Yasinska, I. M., Sakhnevych, S. S., Fiedler, W., Wellbrock, J., Bardelli, M., Varani, L., Hussain, R., Siligardi, G., Ceccone, G., Berger, S. M., **Ushkaryov, Y. A.**, Gibbs, B. F., Fasler-Kan, E., and **Sumbayev, V. V** (**July 2017**)**.** 'The Tim-3-galectin-9 Secretory Pathway is Involved in the Immune Escape of Human Acute Myeloid Leukemia Cells.' *EBioMedicine* 22: 44-57. doi: https://doi.org/10.1016/j.ebiom.2017.07.018

[R2] Sakhnevych, S. S., Yasinska, I. M., Bratt, A. M., Benlaouer, O., Gonçalves Silva, I., Hussain, R., Siligardi, G., Fiedler, W., Wellbrock, J., Gibbs, B. F., **Ushkaryov, Y. A.,** and **Sumbayev, V. V**. (**June 2018**). 'Cortisol facilitates the immune escape of human acute myeloid leukemia cells by inducing latrophilin 1 expression'. *Cellular and Molecular Immunology* 15(11): 994-997. doi: https://dx.doi.org/10.1038/s41423-018-0053-8

[R3] Yasinska, I. M., Sakhnevych, S. S., Pavlova, L., Teo Hansen Selnø, A,, Teuscher Abeleira, A. M., Benlaouer, O., Gonçalves Silva, I., Mosimann, M., Varani, L., Bardelli, M., Hussain, R., Siligardi, G., Cholewa, D., Berger, S. M., Gibbs, B. F., **Ushkaryov, Y. A.**, Fasler-Kan, E., Klenova, E., and **Sumbayev, V. V.** (**July 2019**). 'The Tim-3-Galectin-9 Pathway and Its Regulatory Mechanisms in Human Breast Cancer'. *Frontiers in Immunology* 10: 1594. doi: https://doi.org/10.3389/fimmu.2019.01594

[R4] Sumbayev, V. V., Gonçalves Silva, I., Blackburn, J., Gibbs, B. F., Yasinska, I. M., Garrett, M. D., Tonevitsky, A. G., **Ushkaryov, Y.** (**June 2016**)**.** 'Expression of functional neuronal receptor latrophilin 1 in human acute myeloid leukaemia cells'. *Oncotarget* 7(29): 45575-45583. doi: http://dx.doi.org/10.18632/oncotarget.10039

[R5] Goncalves Silva, I., Rüegg, L., Gibbs, B. F., Bardelli, M., Fruehwirth, A., Varani, L., Berger, S., Fasler-Kan, E., and **Sumbayev, V. V.** (**May 2016**). 'The immune receptor Tim-3 acts as a trafficker in a Tim-3/galectin-9 autocrine loop in human myeloid leukemia cells'. *OncoImmunology* 5(7): e1195535. doi: http://dx.doi.org/10.1080/2162402X.2016.1195535

[R6] Yasinska, I. M., Ceccone, G., Ojea-Jimenez, I., Ponti, J., Hussain, R., Siligardi, G., Berger, S., Fasler-Kan, E., Bardelli, M., Varani, L., Fiedler, W., Wellbrock, J., Raap, U., Gibbs, B. F., Calzolai, L., and **Sumbayev, V. V.** (**February 2018**). 'Highly specific targeting of human acute myeloid leukaemia cells using pharmacologically active nanoconjugates'. *Nanoscale* 10(13): 5827-5833. doi: https://doi.org/10.1039/C7NR09436A

Patents

[P1] Sumbayev, Vadim, Ushkaryov, Yuri, and Gibbs, Bernhard (**2015**). 'Latrophilins as Novel Biomarkers for leukaemia diagnostics'. WO2016203031A1 2016. http://kar.kent.ac.uk/53391

Research grants

[G1] Sumbayev, Vadim. 'A Fundamentally Novel Strategy for the Diagnosis and Treatment of Acute Myeloid Leukaemia'. Medial Research Council (MRC), 2015-20. Value: £49,000.

4. Details of the impact (indicative maximum 750 words)

Transforming research strategies and priorities at the European Commission Joint Research Centre, Diamond Light Source Ltd, and the University of Oldenburg

The European Commission's Joint Research Centre, Diamond Light Source Ltd, and the University of Oldenburg have altered their research strategies and priorities as a result of collaborative work carried out with Sumbayev and colleagues on the AML project **[a, b, d]**.

1. *European Commission's Joint Research Centre*

The Joint Research Centre (JRC) is the European Commission's science and knowledge service, which employs scientists to carry out research in order to provide independent scientific advice and support to EU policy. Between 2017 and 2019, the JRC collaborated with Sumbayev on the AML project. Dr Luigi Calzolai, a scientist working on nanobiotechnology at the JRC (Italy), affirms that 'This work had a strong impact on the research priorities of our institution and we invested funding and resources into this work' **[a]**. Calzolai goes on to explain that the JRC designed and provided gold nanoparticles that Sumbayev and his group used to develop functional nanomaterials to specifically recognise human acute myeloid leukaemia cells **[a]**. The collaboration with Sumbayev resulted in several projects on which the JRC is now working. Calzolai confirms that 'These projects are dealing with development of functionalised nanomaterials in order to recognise specific cells and deliver specific compounds into them (starting in 2017 and running until now [November 2020]). This includes also development of novel and advanced techniques for characterisation and design/optimisation of biocompatible functional nanomaterials. This work included financial investments [c. 140,000 euros] and involvement of human resources – two postdoctoral scientists' **[a]**.

2. *Diamond Light Source Ltd*

Sumbayev and colleagues have collaborated with Diamond Light Source Ltd **[b, c]**, which runs the UK's national synchrotron facility. Diamond's circular dichroism Beamline (B23), which enables structural, functional, and dynamic interactions in materials such as proteins, nucleic acids, and chiral molecules to be observed, was used in the AML project to investigate interactions between key proteins involved in the immune evasion pathway. Dr Rohanah Hussain (Senior Beamline Scientist at Diamond) states that 'This work [carried out since 2016] had a significant impact on our research strategy and appeared to be one of the major research highlights of Diamond Light Source during the last two years' **[b]**.

In 2019, Beamline B23 initiated a new project which includes the development of functional non-toxic peptides to target protein kinase C in order to block exocytosis of immunosuppressive proteins by AML and other cancer cells. Research carried out by Sumbayev led Diamond Light to start the project and revise their research plans **[b]**. Hussain confirms that 'As a result of knowledge obtained via Dr Sumbayev's research, we started a fundamentally novel project here at Diamond Light Source Ltd. We have been developing peptides for the purpose of blocking the enzyme called protein kinase C (PKC) which facilitates exocytosis. Dr Sumbayev's research demonstrated that PKC is involved in the latrophilin-1 triggered immune evasion pathway operated by human cancer cells' **[b]**. Diamond has already invested around £60,000 into the new project to cover the costs of peptide design and characterisation of their ability to bind and inhibit PKC. Hussain explains how the long-term idea is to design cell-penetrating peptides to block the immune evasion pathway, allowing cytotoxic lymphoid cells to display maximal anti-cancer activity **[b]**.

3. The University of Oldenburg

Dr Bernhard Gibbs, Principal Research Associate and Reader in Experimental Allergology at the University of Oldenburg in Germany, has collaborated with Sumbayev and colleagues on research into acute myeloid leukaemia since 2013. Dr Gibbs provided primary human blood samples obtained from healthy donors. He also shared his expertise on the use of primary human blood samples and helped with the ethics documentation. Dr Gibbs confirms that 'the research work of Dr Vadim Sumbayev, to which our institution has contributed, had a significant impact on our research strategy' **[d]**. As a result of the discovery of the latrophilin-1-induced immunosuppressive pathway operated by AML cells **[R1-R6]**, Gibbs confirmed in 2020 that the Division of Experimental Allergology and Immunodermatology have revised their research strategy to include the role of immunosuppressive pathways in cancer and autoimmune diseases. Furthermore, Gibbs confirmed that 'the role of this pathway in AML and other cancers is also being considered as a new research topic in one of our Faculty research priority areas ("Potentialbereich Onkologie"), which is currently being established' **[d]**.

Facilitating changes in medical screening procedures

The findings of the Kent research team and collaborators have had a significant impact on the medical screening procedures at two hospitals. The University Medical Centre Hamburg-Eppendorf (Germany) and the University Hospital of Bern (Inselspital), Switzerland, have changed their sample screening practice for patients with leukaemia and paediatric tumours **[e, f]**. Professor Walter Fiedler (MD) from the University Medical Centre Hamburg-Eppendorf states: 'As a result of our collaborative research [with Sumbayev since 2017] our department amended patient sample screening procedures detecting latrophilin-1 and galectin-9 in them in order to characterise the disease' **[e]**. In 2019, Inselspital Bern also amended their screening procedures of paediatric tumour samples in the hospital as a result of collaborative work led by Sumbayev. Dr Elizaveta Fasler-Kan from the Department of Paediatric Surgery explains that they have found that galectin-9 and its receptor Tim-3 are highly present in paediatric tumours, including, but not limited to, Wilms tumours. Fasler-Kan states: 'Currently, we are screening paediatric tumour samples for expression/levels of Tim-3 and galectin-9 in order to estimate their ability to escape immune surveillance' **[f]**.

Informing teaching programmes for medical and biomedicine students

Two universities (Oldenberg and Basel) and a medical school (Inselspital, Bern) have changed their education programmes for students to include the findings of the Kent team's research **[d, f, g]**.

Professor Gibbs at the University of Oldenburg confirms that 'the research work of Dr Vadim Sumbayev […] has had a significant impact on our […] teaching programmes' **[d]**. Gibbs describes how the Division of Experimental Allergology and Immunodermatology has revised its teaching programmes on immunology and anti-cancer immunity to include the latrophilin-1-induced immunosuppressive pathway, giving the example of latrophilin-1 as a potential biomarker for AML diagnostics **[d]**. Gibbs states: 'This includes undergraduate medical students doing electives in

our Division as part of "Longitudinales Forschungscurriculum" (which took place in both summer and winter semesters since 2019), as well as a new international M.Sc. course, which has just started, in Molecular Biomedicine (currently involving 14 students)' **[d]**.

Sumbayev's discovery is also having an impact on the teaching programmes for Biomedicine students at the University of Basel (Switzerland) **[g]**. Dr Fasler-Kan confirms that 'Each year group includes 100 students and since 2018 we have included the Tim-3-galectin-9 immune evasion pathway into the Basic Immunology course when covering aspects of anti-tumour immunity and immune evasion pathways' **[g]**. Inclusion of Sumbayev's findings is helping students to better understand the severity of blood cancers and the inability of the immune system to deal with them **[g]**. Fasler-Kan explains how teaching about the pathway helps to illustrate the power of anti-cancer immune evasion machinery to impair the activity of immune competent lymphocytes leading to disease progression **[g]**. Publications from the Kent team and a sound recording of a webinar given by Sumbayev at the 23rd World Congress on Advances in Oncology in 2018 are used as teaching materials on the course **[h]**.

Dr Fasler-Kan also states that the research work of Sumbayev and colleagues has influenced teaching programmes and led to the inclusion of Kent's discovery in teaching programmes for medical students at Inselspital Bern **[f]**. Fasler-Kan confirms that 'we have included [Sumbayev's] findings in our Advanced Immunology programme for medical students (undergraduate) – roughly 100 students' **[f]**. Kent's research is helping students at Inselspital to understand the concepts of tumour immune evasion and tumour associated antigens. Fasler-Kan also confirms that students are being taught about latrophilin-1 as an example of a biomarker that is expressed in the cancer cells of the vast majority of AML patients **[f]**.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[a] Corroborating statement from the Joint Research Centre (JRC), European Commission, Italy, evidencing the impact on research priorities of the JRC.

[b] Corroborating statement from Diamond Light Source, UK, evidencing the impact on research strategy.

[c] Diamond Light Source's Annual Reviews from 2017-18 and 2018-19, describing collaborative work carried out with Sumbayev and colleagues on Beamline B23.

[d] Corroborating statement from the University of Oldenburg, School of Medicine, Germany, evidencing the impact of the Kent team's research strategy and teaching programmes.

[e] Corroborating statement from the University Medical Centre Hamburg-Eppendorf, Germany, evidencing changed sample screening.

[f] Corroborating statement from the University Hospital, Bern (Inselspital), Switzerland, evidencing the impact of the Kent team's research on teaching programmes and changed sample screening.

[g] Corroborating statement from the University of Basel, Switzerland, evidencing the impact of the Kent team's research on teaching programmes.

[h] Sound recording of a webinar given by Sumbayev at the 23rd World Congress on Advances in Oncology in 2018. The recording is used as teaching material by Dr Fasler-Kan at the University of Basel to illustrate the power of anti-cancer immune evasion.