

Grant Impact Report

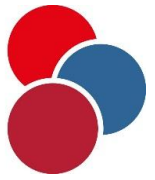
Name	Rhys Morgan
Job title	Research Fellow
Organisation	University of Bristol, School of Cellular & Molecular Medicine
Co-investigators (if applicable)	Dr. Allison Blair Prof. Ann Williams
Grant awarded	Early-Stage Research Start-up Grant: <i>Characterising the β-catenin interactome in primary acute myeloid leukaemia blasts</i>
Year awarded	2016
Date started	1/03/2017
Date completed	25/01/2018
Total amount expended (£)	£10,000

This is the BSH grant impact report form. Please enter the full grant details above, and fill out the form below. The form should be completed electronically and sent to grants@b-s-h.org.uk. Please note that the report can only be accepted if all sections have been completed in full.

In addition: Please include a recent photo of yourself.

Your grant report and photo may be published in our communications materials, including our website and social media platforms.

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1. Please summarise what the grant enabled you to achieve; what would not have been possible without the funding? (Up to 500 words)

Wnt/ β -catenin signalling is an evolutionary conserved signalling pathway critical for normal development and heavily implicated in human cancer. The central mediator, β -catenin, is frequently overexpressed in acute myeloid leukaemia (AML) where its expression is linked with inferior patient survival. Furthermore, well characterised experimental models of AML development have demonstrated β -catenin to play a pivotal role in leukaemogenesis, rendering the protein an ideal therapeutic target.

The stability, subcellular localization and transcriptional activity of the central mediator β -catenin is dictated heavily by proteins interactions. To date, much of its molecular characterisation has been performed in epithelial cells, however β -catenin's haematopoietic interactome is likely to vary from its epithelial interaction network given its less prominent cell adhesion role (adherens junctions) in blood cells, and the relative scarcity of activating Wnt mutations (*APC*, *Axin* and *β -catenin*) which are frequent in solid tumours, but rare in haematological malignancy.

Pertinent to the molecular targeting of β -catenin to disrupt its pathological role in AML, is the prior characterisation of its interacting partners in leukaemia cells. My BSH early-stage research start up grant has allowed me to assess the feasibility of performing β -catenin interactome studies in leukaemia cells for the first time. Specifically, we have trialled the process of co-immunoprecipitating (co-IP) β -catenin protein from both leukaemia cell lines and primary AML patient blast samples, followed by mass spectrometry analysis.

Obtaining research funds from major grant funding bodies would have been a considerable challenge given the perceived level of risk associated with our experimental approach. As a junior research scientist, I would have also struggled in more competitive funding schemes given my relative inexperience compared with more senior co-applicants in the field.

However, our now optimised experimental strategy has proved successful and the BSH grant has provided me with the novel and interesting data I require to build a more substantial and long-term research bid.

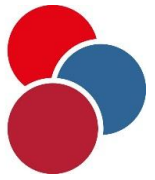


2. Briefly describe the aims and expected outcomes of this project. (Up to 300 words)

The two main aims of the project, as stated in my original research proposal, were as follows:

1. To assess the feasibility of performing β -catenin Co-immunoprecipitation (co-IP) from primary AML patient blast samples, followed by the use of Tandem Mass Tagging (TMT) coupled to LC-MS/MS-based quantitative proteomics to identify novel β -catenin interacting proteins.
2. To identify and validate novel candidate interacting proteins/complexes of potential importance to β -catenin level/activity/localisation in AML blasts.

Although, there was some indication from pilot studies that the above experimental approach was effective in leukaemia cell lines, the method was thus far untested on AML patient samples. The expectation was this experimental strategy would work well using primary AML tissue (exhibiting high β -catenin expression) and would validate interactions detected in leukaemia cell lines that could have clinical relevance.



3. Describe the key outcomes to date. Please summarise your conclusions. (Up to 600 words)

This study has achieved the following key outcomes:

1. This project has definitively demonstrated that it is technically feasible to efficiently co-IP β -catenin protein from both leukaemia cell lines and primary AML blasts (exhibiting high β -catenin expression). Furthermore, it has shown that mass spectrometric analysis of these co-IP's using quantitative, high resolution proteomic technologies is capable of characterising β -catenin protein interactions in both the cytosol and nucleus of leukaemia cells. This experimental strategy, plus the proceeding bioinformatics analyses, has been validated through the frequent detection of known established β -catenin interactions such as TCF4, LEF1, APC, Axin, α -catenin, CK1, GSK3 β and BCR-ABL in leukaemia cells.
2. The purpose of characterising β -catenin's protein interaction network in AML cells is to identify potential clinically relevant interactions which may regulate its stability, localisation and activity in leukaemia cells. In this study, we been able to identify a protein that, in addition to mediating β -catenin's transcriptional activity, can also modulate its nuclear localisation in leukaemia cells (manuscript in preparation). Using lentiviral transduction of leukaemia cell lines, we have shown that knockdown of this protein in Wnt responsive cell lines (K562 and HEL) significantly reduces the amount of nuclear β -catenin that can be nuclear localised. Furthermore, we have reciprocally shown that overexpression of this protein in Wnt unresponsive cell lines (that induce poor levels of nuclear β -catenin) can markedly increase levels of nuclear β -catenin, leading to Wnt signalling activation.
3. Finally, this study has identified a number of novel and exciting β -catenin protein interactions that may have clinical interest and thus represent attractive targets for further study. A number of these proteins are already reported overexpressed and/or mutated in AML raising the fascinating prospect that β -catenin synergises with other molecular aberrations in AML. A number of these interacting proteins are completely novel to both the haematological and Wnt signalling fields, providing a unique opportunity to characterise their role in blood cells for the first time. Pilot studies will now continue in my laboratory to validate the most interesting interactions and establish their biological significance in both leukaemia, normal haematopoietic development and Wnt signalling overall. In addition to this, hundreds of other interactions have been discovered which will form the basis of small studies for undergraduates or placement students who join my lab for short projects.

In summary, this study has demonstrated that it is technically feasible to efficiently co-IP β -catenin from primary AML blasts, and subsequently use these samples to identify β -catenin protein interactions of clinical interest for further study.



4. List published papers, oral and/or poster presentations as a result of this grant. Include manuscripts in preparation or in submission / under review, prefaced by an asterisk.

β -Catenin interactome profiling of myeloid leukaemia cells reveal novel context dependent protein partners. Ridsdale J, Shao R, Heesom K, Wilson M, Greenhough A, Williams AC, Blair A, Tonks A, Darley RL, Morgan RG. *Poster presentation at Gordon Research Conference – Wnt Signaling: A Pathway Implicated in Animal Development, Stem Cell Control and Cancer. August 6-11, 2017. Stoweflake Conference Center, Stowe, VT, USA. (Poster attached).*

β -Catenin interactome studies reveal X as an important regulator of β -catenin nuclear localization in myeloid leukemia cells. Morgan RG, Ridsdale J, Shao R, Heesom KJ, Wilson MC, Davidson AD, Greenhough A, Williams AC, Blair A, Waterman ML, Tonks A, Darley RL. *Manuscript in preparation, due for submission to Leukemia in February/March 2018 (BSH funding duly acknowledged in manuscript).*

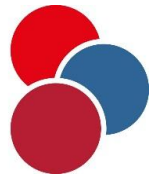
5. Did any patent applications arise from this work? (If yes, please detail. Up to 200 words)

No patent applications were generated from this project.

6. Were you successful in any further grant applications as a result of this work? (If yes, please detail. Up to 200 words)

No further grants have been applied for at this point, however, the project has yielded a considerable amount of novel data from which many tens of research proposals will be generated. Specifically, I am planning to use the novel data generated from this project to form the basis of a *New Investigator Research Grant* (NIRG) application to the Medical Research Council.

The grant has also been invaluable in allowing me to secure a lectureship at the University of Sussex. Data generated from BSH funding was used during my interview presentation which was well received by panel members and convinced them of my long-term research ambitions. As part of my start-up package for this post I will receive a PhD studentship, which will be constructed around data generated from this study. This BSH grant has contributed significantly in my ultimate long-term ambition of establishing my own laboratory within the field of leukaemia research.



7. Did new collaborations arise from this work? (If yes, please detail. Up to 400 words)

A number of new and exciting collaborations have arisen from this work which have helped me greatly with the current project and will serve me well going forward with future publications and grant applications.

My attendance at the Wnt signalling meeting in August 2017 allowed me to share my BSH funded data with Professor Marian Waterman of University of California, Irvine, CA, USA. Prof. Waterman is an international expert in the Wnt signalling pathway and was enthused by the novel data I presented. Subsequently we have shared multiple Skype meetings over the past 6 months where she has helped raise my work to publication standard.

This study has generated a considerable amount of bioinformatics data for which I have required help to properly analyse. For this, I have sought the services of Dr. Rob Ewing from Biological Sciences, University of Southampton, who has been instrumental in providing bioinformatics support. In addition to this project, we have a further ongoing study which we hope to bring to publication at some point in 2019.

Attendance at the Wnt signalling meeting of 2017 also put me in close contact with Dr. Sebastian Guettler of the Institute of Cancer Research, London. From my poster presentation, Dr. Guettler became very interested in the proteomics approach I adopted to identifying novel β -catenin interactors in leukaemia cells and I have been able to share methodological tips and protocols with him. I have subsequently hosted him in Bristol to give a departmental seminar and form closer links with other PI's in the school.

Finally, the study has strengthened my existing collaboration with the haematology team within the Division of Cancer & Genetics at Cardiff University (Prof. Richard Darley and Dr. Alex Tonks) who's project guidance, supply of patient samples and reagents has been integral to the success of the project. Data from this study has inspired the creation of a potential new GW4-funded PhD studentship at Cardiff University examining research along a similar theme:

<http://www.cardiff.ac.uk/study/postgraduate/research/programmes/project/phd-in-cancer-and-genetics-the-role-of-wnt-signalling-in-blood-stem-cell-development-and-in-acute-myeloid-leukaemia>

8. What was the funding amount you received and how was it actually spent? (detail item/activity and amount spent in pounds)

Received £10,000 from BSH which was spent as below:

Equipment (including Microplate reader and Western blotting apparatus) - **£6,950.10**

IT software - **£70.00**

Consumables - **£391.77**

University of Bristol Proteomics Facility costs - **£1,820.92**

Travel costs to meet with collaborators and present novel data at Wnt signalling conference - **£760.22**

Total - £9,993.01

9. What are the future research priorities in this area?

Having generated the first interactomes for β -catenin in leukemia cells, and identified a plethora of potential novel interactors, the priority for this field must now be to establish the biological significance of novel β -catenin protein interactions in the context of AML pathology. Candidates to be taken forward for further investigation and potential grant funding will be shortlisted according to their novelty, specificity to leukaemia and availability of research tools (antibodies, expression constructs, etc). Upon obtaining further grant funding it will be vital to characterise how the interaction affects the pathology of leukaemia cells (survival, proliferation, drug sensitivity etc) and indeed overall Wnt signalling output. If pathological phenotypes of any interaction can be defined, then further study will be necessary, in collaboration with industry/pharma, to assess the pharmacological capability of disrupting β -catenin interactions for therapeutic intervention in leukaemia.

Finally, I wish to thank the BSH whole heartedly for awarding me this grant and allowing me to generate the pilot data necessary to both realise my research ambitions and enhance my future scientific career.